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Seasonal and Diel Patterns of Abundance and Productivity of Phototrophic Picoplankton in the Lower Chesapeake Bay

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SEASONAL AND DIEL PATTERNS OF ABUNDANCE AND PRODUCTIVITY OF PHOTOTROPHIC PICOPLANKTON IN THE LOWER CHESAPEAKE BAY

by

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ABSTRACT

Seasonal and Diel Patterns of Abundance and Productivity of Phototrophic Picoplankton in the Lower Chesapeake Bay

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Old Dominion University, 1990
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This study was performed to evaluate phototrophic picoplankton (0.2 to 2.0 μm) dynamics within the lower Chesapeake Bay. A 15 month study of phototrophic picoplankton abundance and productivity was made from June 1988 to October 1989. Annual picoplankton abundance using epifluorescence microscopy ranged from 7.26×10^6 cells/l in the winter to 9.28×10^8 cells/l during late summer.

In situ incubations of natural picoplankton populations over the 15 month study were used to test the applicability of the frequency of dividing cells technique in estimating phototrophic picoplankton growth rates. The regression equation of $\mu = 2.37 \times 10^{-3}$ (FDC) + 0.024 was developed to estimate phototrophic picoplankton growth rates in the lower Chesapeake Bay where productivity values were estimated using phototrophic picoplankton abundance and carbon content. Limitations and improvements in using the frequency of dividing cells technique were discussed. Productivity estimates using both

frequency of dividing cells and sodium ^{14}C-bicarbonate fractionation techniques identified phototrophic picoplankton contributing over 50% of total primary productivity during the summer season.

Two high frequency diel studies measuring phototrophic picoplankton abundance and productivity in summer and winter seasons revealed physical factors in the water column partly determining phototrophic picoplankton distribution. Higher phototrophic picoplankton concentrations were associated with waters seasonally above the pycnocline. In summer, phototrophic picoplankton concentrations were highest during ebb tide when the dominant phototrophic picoplankton was phycocyanin enriched *Synechococcus* sp. In winter, phototrophic picoplankton concentrations were highest during flood tide when phycoerythrin enriched *Synechococcus* sp. dominated phototrophic picoplankton composition. Availability of phototrophic picoplankton carbon within the water column is discussed as to its influence to Bay trophodynamics.

DEDICATION

To my parents

ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

INTRODUCTION

Photoautotrophic picoplankton (0.2 to 2.0 μm) represent the most abundant and productive plankton component in the marine environment (Li et al., 1983; Marshall and Lacouture, 1986; Smith et al., 1985). However, there are many unanswered questions concerning their role in the marine ecosystem. These questions include information on their growth rate, metabolism, linkage between trophic levels and their status as a sink for nutrients (Ducklow et al., 1986; Sherr et al., 1987). A first step to understand the picoplankton role in the marine environment is to interpret picoplankton population dynamics on time scales which correspond to this microscopic component. Information on fluctuations of picoplankton abundance within the water column in relationship to changes in various physical factors would also provide information as to the conditions that would influence the availability of the picoplankton as a link or sink in estuarine trophodynamics.

The objectives of this study are as follows: 1) Develop a relationship between frequency of dividing cells (the number of cells that are undergoing cell division) and μ (growth rate) from *in situ* incubation studies to use as a tool for

estimating productivity for the photoautotrophic component of natural picoplankton populations in the lower Chesapeake Bay, 2) identify and describe seasonal patterns of abundance and productivity of photoautotrophic picoplankton at the entrance of the Chesapeake Bay, 3) identify the contribution of photoautotrophic picoplankton productivity to total productivity at the entrance of the Chesapeake Bay, 4) identify and describe diel patterns of abundance and productivity of photoautotrophic picoplankton in relation to physical characteristics of the water column at the entrance of the Chesapeake Bay, and 5) compare abundance and productivity dynamics of photoautotrophic picoplankton over a 24 hour study period in the winter and summer seasons.

Definition and history

Picoplankton is defined as that component of the plankton between 0.2 and 2.0 μm in size (Sieburth et al., 1978). There are a variety of both heterotrophic and autotrophic organisms included in the picoplankton classification of marine systems (Hanson et al., 1983; Johnson and Sieburth, 1982). Originally, the term "picoplankton" was used as a collective term to identify both heterotrophic and autotrophic organisms that could pass through a 2.0 μm filter. Microbiologists prefer to identify the heterotrophic component of picoplankton as "bacterioplankton" (Fuhram et al., 1980; Hagström et al., 1984). As a result of this distinction of

heterotrophic forms, picoplankton is more commonly used to denote only those photoautotrophic organisms found in the picoplankton size range of aquatic systems that fix carbon by photosynthesis using chlorophyll and accessory pigments (Johnson and Sieburth, 1982). Autotrophic picoplankton is composed of a variety of both eukaryotic and prokaryotic forms including the coccoid cyanobacterium *Synechococcus* (Thomsen, 1986; Waterbury et al., 1986). For this study, the term "picoplankton" will denote only those photoautotrophic organisms that fall within the size range of picoplankton as defined by Sieburth et al. (1978).

One of the earlier reports of this microscopic component was made by Lohmann (1911) while studying the feeding behavior of appendicularians. Rodhe (1955) observed "μ-algae" in freshwater subartic lakes in Sweden. Paerl (1977) used the term "little green things" tc describe this unknown phytoplankton component in lake studies from New Zealand. Some investigators have data describing the existence of an active photosynthetic component in the <3 micron size range in fractionation work in marine environments (Saijo, 1964). The first reports of unicellular cyanobacteria being present in great numbers in oceanic systems were made by Johnson and Sieburth (1979) and Waterbury et al. (1979). Unfortunately, there are difficulties and limits as to the resolution in identifying this microscopic component using light microscopy

(Pirie, 1964). As a result, much of the earlier investigations either ignored or improperly described this picoplankton component. It was not until the use of fluorescence microscopy that an accurate account of this picoplankton component could be determined. Fluorochromes were used to distinguish heterotrophic organisms from photoautotrophic forms making identification and counting more reliable (Daley and Hobbie, 1975).

Picoplankton abundance and productivity

The use of fluorescence techniques brought about an extensive amount of research investigating the distribution of picoplankton in both marine and freshwater systems throughout the world (Caron et al., 1985; Chang, 1980; Craig, 1985 Cronberg and Weibull, 1981; Fisher, 1985: Hallegroeff and Jeffrey, 1984; Johnson and Sieburth, 1979; Krempin and Sullivan, 1981; Li et al., 1983; Smith et al., 1985; Takahashi and Hori, 1984). There are numerous studies showing the picoplankton component of phytoplankton being widespread and comprising the majority of phytoplankton abundance and total cell volume in many marine environments (Johnson and Sieburth, 1979; Li et al., 1983; Takahashi and Bienfang, 1983; Takahashi and Hori, 1984; Waterbury et al., 1979). From a study of phototrophic ultraplankton, Murphy and Haugen (1985) have suggested cyanobacterial abundance declines with decreasing temperature and northerly increasing latitude. In waters off Oahu Hawaii, 60% to 80% of microbial biomass occurred in the

< 3 μm fraction (Bienfang et al., 1984). In the coastal waters of Japan, the percentage of picocyanobacteria (picoplankton) biomass (cellular carbon) ranged between 8.3% to 79.4% of the total picophytoplankton and between 4.7% to 46.7% of the total phytoplankton (Takahashi et al., 1985).

Autotrophic picoplankton is one of the most abundant and productive planktonic components in our marine environment (Li et al., 1983; Marshall and Lacouture, 1986; Smith et al., 1985). Specific growth rates of marine picoplankton have been reported by Douglas (1984) to be as high as 8.9 day^{-1}. Stockner and Antia (1986) reported picoplankton carbon production ranging from 0.01 $mgC \cdot m^{-3} \cdot hr^{-1}$ to 31 $mgC \cdot m^{-3} \cdot hr^{-1}$ from a variety of aquatic habitats. Several studies have revealed picoplankton being responsible for over 50% of total primary productivity in marine environments (Platt et al., 1983; Li et al., 1983; Iturriaga and Mitchell, 1986; Takahashi et al., 1985). In a review study of picoplankton by Stockner and Antia (1986), the contribution of picoplankton production to total carbon production ranged between 1% and 90% where higher contributions were reported in oligotrophic oceanic systems. In the North Atlantic Ocean, Platt et al. (1983) reported picoplankton contained a significant autotrophic component capable of supplying about 60% of the total primary productivity in an open ocean ecosystem. Takahashi and Bienfang (1983) observed over 75% of total ^{14}C fixation in studies off Hawaii was due to autotrophic phytoplankton < 3 μm. Li et al.

(1983) reported productivity in the < 1 μm size fraction in the tropical Pacific to vary from 20% to 80%. Joint et al. (1986), from the only study describing seasonal pattern flux of picoplankton in temperate marine waters, noted maximum production rates and relative contribution to total phytoplankton productivity occurred in midsummer. Similar picoplankton growth rates, production values and contribution to total primary production is also found in freshwater systems. Using autoradiographic techniques, Paerl and Mackensie (1977) reported picoplankton assimilating a significant amount of carbon in freshwater lakes of New Zealand. Similar findings of picoplankton productivity in freshwater environments have been reported by Fahnstiel et al. (1986) where 50% of carbon production and chlorophyll biomass is attributable to picoplankton.

Several studies have focused on the vertical distribution of picoplankton productivity and abundance in relation to physical factors influencing the water column (Bienfang et al., 1984; Craig, 1984; Johnson and Sieburth, 1979; Joint, 1986;, Joint and Pomeroy, 1983; Platt et al., 1983; Takahashi and Bienfang, 1983; Waterbury et al., 1979). In a study of the Costa Rica Dome, Li et al. (1983) reported the productivity importance of the picoplankton fraction increased toward the base of the euphotic zone. They attribute the relative enhancement of inorganic carbon uptake in the small size fraction at this depth to the ability of the picoplankton

cells to use relatively dim light efficiently. Both prokaryotic and eukaryotic cells of the autotrophic picoplankton can thrive at low irradiances (Richardson et al., 1983). In the North Pacific and South China Seas, Takahashi and Hori (1984) noted the dominant phytoplankton component in the subsurface chlorophyll maximum layer was picoplankton. Morris and Glover (1981) reported photosynthesis by cells < 1 μm contribute more to productivity at lower light intensities and suggest cyanobacteria are expected to make a significant contribution to photosynthesis at the base of the euphotic zone. Vertical profile data by Iturriaga and Mitchell (1986) indicated the maximum amount of cyanobacteria were associated with the maximum density and fluorescence parameters they measured in the North Pacific Ocean. In studies of the chlorophyll maximum in waters off Hawaii, Bienfang and Szyper (1981) reported 80% of the biomass is associated with picoplankton. Large concentrations of chlorophyll can also be associated with tidal fronts (Pingree et al., 1975). Joint (1986) reported picoplankton have not been attributed to the increase in the chlorophyll maximum along tidal fronts nor have picoplankton been attributed to the chlorophyll maximum associated with the thermocline in temperate waters.

Unfortunately, few studies on picoplankton have focused on abundance and productivity measurements emphasizing temporal and spatial scales comparable to this picoplankton size component. Joint et al. (1986) expressed concern that

many of the temporal scales for sampling this picoplankton component have been too restricted, e.g. due to cruise length. Temporal and spatial scaling is very important if true productivity and abundance patterns are to be recognized (Harris, 1980). High frequency sampling may provide insight to productivity and abundance dynamics not revealed in studies where productivity and abundance values for a marine system are estimated from a single sampling and a short term experimental design.

Techniques for measuring picoplankton productivity

Several investigators have successfully separated picoplankton from nanoplankton and microplankton by differential filtration techniques (Waterbury et al., 1979; Johnson and Sieburth, 1979; Caron et al., 1985). *Synechococcus* sp., a major component of picoplankton, is reported extremely resistant to cell rupture and are able to withstand pressures involved in filtration methodology (Waterbury et al., 1986). A popular method to measure productivity of marine autotrophic picoplankton is with timed incubations using sodium ^{14}C-bicarbonate (Gieskes et al., 1979; Li et al., 1983; Platt et al., 1983; Takahashi and Bienfang, 1983). Waterbury et al. (1986) recommended the use of pre-incubation fractionation techniques as opposed to post-incubation fractionation, as the latter tended to overestimate ^{14}C-

bicarbonate assimilated by *Synechococcus*. This over estimation was hypothesized due to the disruption of eukaryotic cells during filtration. To avoid incubation procedures required by the ^{14}C method and to measure productivity on high frequency time scales, a more direct measure of productivity is available. Frequency of dividing cells is a non-incubation method based on both theoretical and experimental evidence that the frequency of dividing cells of a population is dependent on the population growth rate (μ) (Hagström et al., 1979; Newell and Christian, 1981). Past studies using the FDC (frequency of dividing cells) method to estimate productivity have concentrated on the heterotrophic population, or a combination of the heterotrophic and autotrophic populations in marine systems (Davis and Sieburth, 1984; Christian et al. 1982; Hagström et al., 1979; Hanson et al., 1983; Newell and Christian, 1981).

In past studies utilizing the FDC technique, the established relationship between FDC and μ used to estimate productivity focused on *in vivo* culturing experiments over limited temperature regimes (Christian et al., 1982; Hagström et al., 1979; Newell and Christian, 1981; Campbell and Carpenter, 1986). Some investigators have indicated these laboratory incubations alter the growth characteristics of natural assemblages, not giving a true indication of growth rates in natural environments (Hanson et al., 1983). Another

criticism of the FDC method is the effect of bacteriovores on the growth characteristics of natural picoplankton populations (Hanson et al., 1983).

Work by Campbell and Carpenter (1986) revealed natural *Synechococcus* populations in the Sargasso Sea have a diel pattern in their frequency of dividing cells. To calculate growth rates of these picoplankton populations, Campbell and Carpenter (1986) used a mathematical equation describing phytoplankton growth as derived from McDuff and Chrisholm (1982). This non-incubation procedure to estimate growth rate revealed a strong correlation with instantaneous daily growth rates calculated from on-deck incubation experiments (Campbell and Carpenter, 1986). In this study, consideration was given to the importance of measuring the duration of picoplankton cell division and its effects on the FDC technique in estimating picoplankton productivity.

Role of picoplankton in the marine environment

Reported observations of high productivity and abundance of picoplankton occurring in marine systems have led to controversy as to the role of this component. Ducklow et al. (1986), using mesocosm experiments, found picoplankton to be a "sink" for carbon in planktonic food webs. Sherr et al. (1987) disagreed with this "sink" explanation, and described the picoplankton component as a link, or food source for

higher trophic levels. This concept was supported by numerous studies emphasizing the relationships of grazing microprotozoa on the picoplankton component (Anderson and Fenchel, 1985; Laval-Peuto et al., 1986; Porter et al., 1985; Rassaulzadegan and Sheldon, 1986). Estep et al. (1986) reported the existence of nanoflagellates in many marine environments. This finding of algal nanoflagellates complicates the processes involved in the microbial food loop, since protists with and without chloroplasts can actually occupy overlapping trophic levels. Another area of the marine environment where picoplankton serve as a food source is the benthic environment, where suspension and deposit feeders predominate. Unfortunately, much of the microbial information on the carbon flow and nutrient recycling dynamics in the benthos has focused on heterotrophic bacteria (Fenchel and Jorgensen, 1977; Meyer-Reil and Faubel, 1980).

In addition to their biological influence on the marine ecosystem, picoplankton can potentially influence the physical and chemical characteristics of the marine environment. Because of their high growth rates, these autotrophic organisms can, through respiration and decomposition, contribute to hypoxic and anoxic conditions in shallow estuarine environments. Certain species of picoplankton, including *Synechococcus*, have the capability to fix diatomic nitrogen *in vivo* (Mitsui et al., 1986; Waterbury et al., 1988). Because picoplankton are

so numerous and productive, these findings have major implications in the biogeochemical cycling dynamics of a very important and limiting element in estuarine and marine environments.

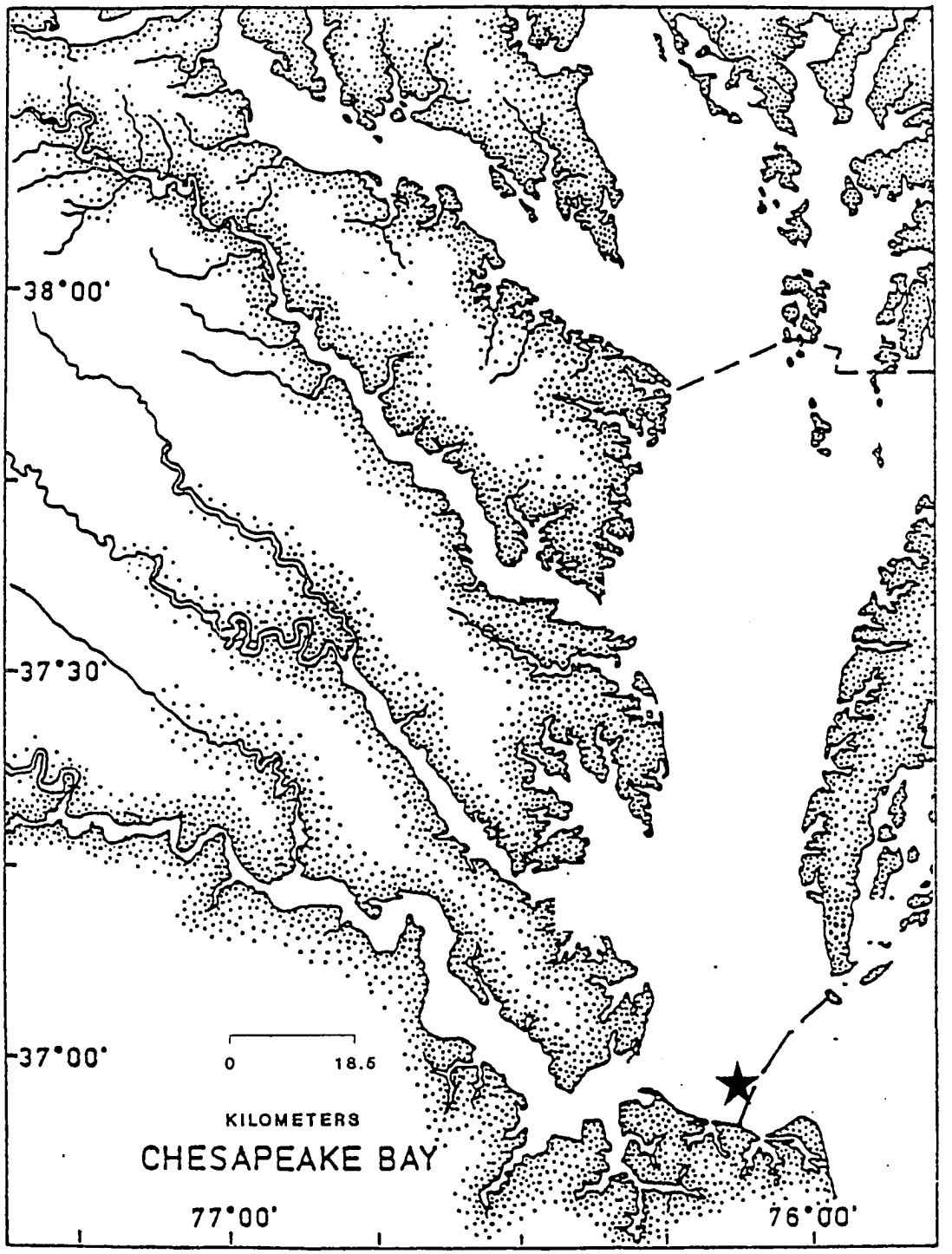
Chapter 2

SITE DESCRIPTION

The sampling site is located at the entrance of the Chesapeake Bay between Cape Charles and Cape Henry. More specifically, the sampling site is situated on the northern end of a fishing pier located on the south island of the Chesapeake Bay Bridge Tunnel complex (Figure 1). The fishing pier is 4.8 kilometers north from the southern point of the Chesapeake Bay Bridge Tunnel and is adjacent to Thimble Shoals Channel. At mean high water, the depth of the water column is seven meters with an average tidal range of 0.46 meters (NOAA, 1989b). This particular site was chosen for accessibility to a stationary platform necessary to enable consistent and high frequency sampling required for this study.

The proximity of the lower Bay system to the Atlantic Ocean provides a more stable environment compared to the upper reaches of the Bay and its tributaries. In general, salinities are lowest during the spring (February through April) and highest in the summer and fall when there is a lower river discharge (Pritchard, 1952). There is far less variability in the temperature and salinity regimes in the lower Bay compared to the tributary environments. Much of the character

Figure 1. Map of Chesapeake Bay showing sampling station.



of the lower Bay system is dependent on the exchange of water from the Atlantic shelf system. There is also subpycnocline movement of more saline waters along the bottom portion of the Bay system. Tyler and Seliger (1978) reported a net transport of bloom producing phytoplankton being carried toward the upper reaches of the Bay system in the more saline bottom waters. Conversely, the phytoplankton of coastal and shelf waters are influenced by the outflow of lower saline waters from the Chesapeake Bay (Marshall, 1981). Frequent plume formations transport materials from the Bay entrance southward along the Atlantic coast (NASA, 1981). In studying the halocline structure of the lower Chesapeake Bay, Heltzel (1973) found the halocline structure to vary seasonally with a greater salinity-depth gradient average noted in summer, and a minimum salinity-depth gradient average in winter. Effects on halocline structure in the lower Chesapeake Bay were attributed to the influence of river runoff (Heltzel, 1973).

Chapter 3

MATERIALS AND METHODS

Preliminary experiment to test FDC validity

To test the applicability of the Frequency of Dividing Cells technique for estimating growth rates of the phototrophic picoplankton, a separate laboratory incubation was run as a control. The incubation involved inoculating one 300 ml glass incubation bottle containing 100 ml of culture media (medium #617 from the American Type Culture Collection) with a pure culture of *Synechococcus* sp. (#27265 from the American Type Culture Collection). The original culture of *Synechococcus* sp. was grown in an aerated one liter glass bottle containing 750 ml of culture media. It was maintained over a three week period prior to this experiment in natural light - dark conditions in the Old Dominion University Greenhouse. After a day of acclimation in sunlight, the incubation bottle was placed on a shaker apparatus to mix the culture. Incubation was carried out at room temperature throughout the daylight and extended approximately one hour after sunset (eight hours total). Three replicate samples were taken approximately every three hours over the incubation period. The samples were fixed in glutaraldehyde (1% final concentration) and

stained using DAPI (4', 6-diamidino - 2 phenylindole). Total *Synechococcus* abundance and the frequency of dividing cells were enumerated using epifluorescence microscopy (see section on enumeration procedures). From plots of *Synechococcus* abundance and FDC over time, validity of the FDC technique was determined by following patterns of FDC in relation to growth patterns of the *Synechococcus* population.

Incubation procedures for FDC and μ

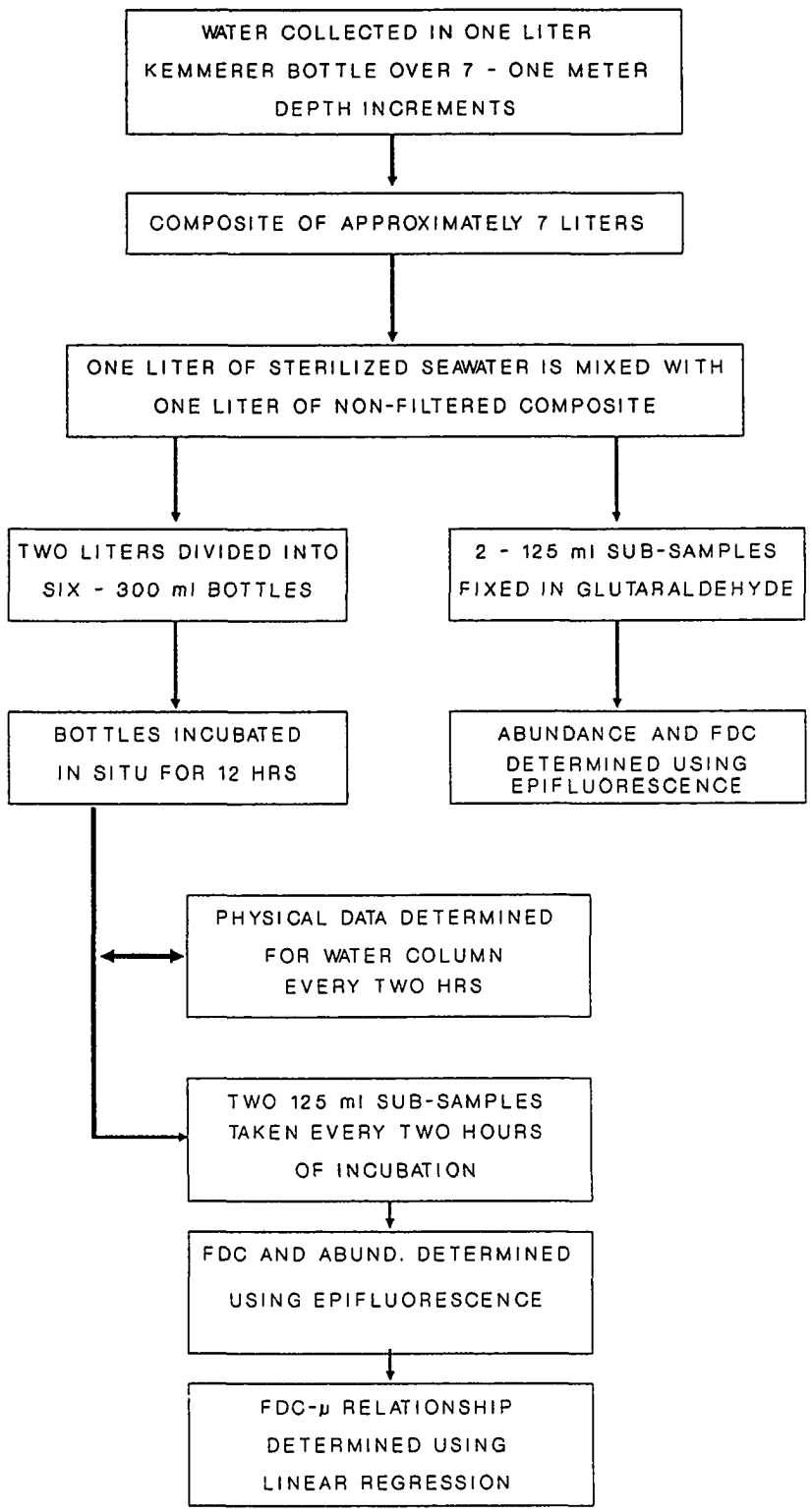
To establish the necessary relationship of FDC and μ for autotrophic picoplankton, a total of 15 *in situ* incubations were performed for this portion of the study at the pier site on the south island. In 1988, incubations were performed in June, July, September, October, November, and December; in 1989, February, March, April, May, June, July, August, September, and October. This range of incubations provided a variety of temperature, nutrient and light intensity conditions in relation to picoplankton populations in the water column.

All glassware used in this study was soaked for 24 hours in HNO_3 (0.5 N) and triple rinsed with distilled water to remove effects from metal ion interference on productivity (Fitzwater et al., 1982). Water for the composite sample was collected from the northeast side of the fishing pier using a one liter Kemmerer Bottle. The composite water sample was

collected over one meter increments throughout the water column (7 m) and placed in a 20 liter plastic carboy (Figure 2). One liter of filter sterilized seawater (seawater passed through a 0.2 μm Nucleopore filter) was mixed with one liter of unfiltered composite sample. By diluting the composite sample, competition for nutrients by some larger phytoplankters and grazing pressure by zooplankton should be reduced. In addition, a diluted sample would allow picoplankton cells to enter an exponential phase of growth. Two 125 ml subsamples of the diluted composite were placed in Nalgene plastic sampling bottles containing glutaraldehyde (1% final concentration) and returned to the Old Dominion University Phytoplankton Laboratory for enumeration using epifluorescence microscopy. An average cell count from the two samples collected represented the abundance of picoplankton at the start of incubation (t_0).

Subsamples from diluted composite sample were placed in six 300 ml incubation bottles and allowed to incubate one meter below the water's surface for approximately 12 hours (actual incubation time depended on the light period of the season (9.6 to 14.75 hours). Secchi depth, temperature, and salinity readings were taken at the depth of incubation approximately every two hours using a thermometer, refractometer and secchi disk (9 in diameter). Every two hours of the incubation, two 125 ml subsamples were taken from one of

Figure 2. Sampling and procedural protocol.



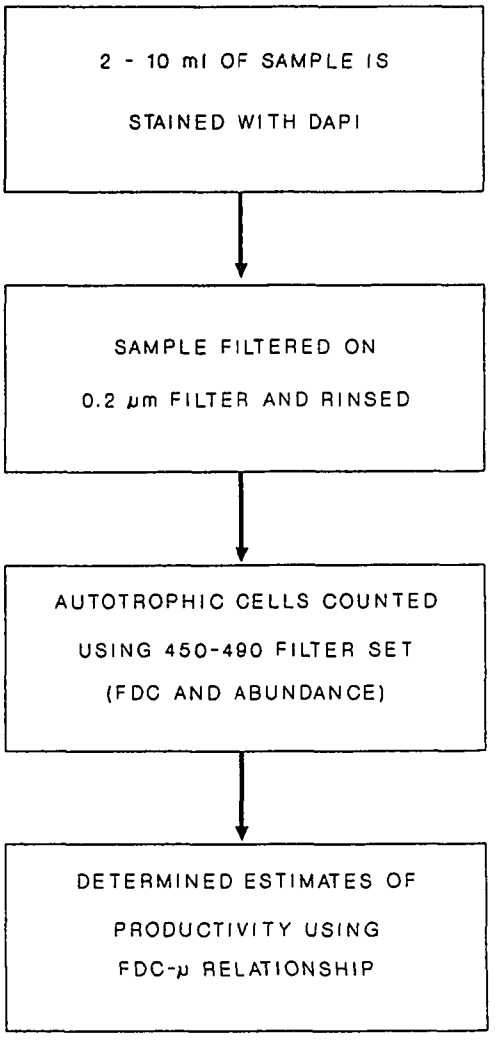
the six incubation bottles, and fixed in glutaraldehyde (1% final concentration) for enumeration and FDC counts.

Enumeration procedures

For enumeration, DAPI (4',6-diamidino-2 phenylindole) was used as the fluorochrome for staining the picoplankton component (Porter and Feig, 1980). A subsample (2 to 10 ml, depending upon cell density) was taken from the sample and incubated in the dark with 100 µl/ml of DAPI fluorochrome for seven minutes. After incubation, the picoplankton-fluorochrome sample was filtered on a 0.2 µm Nucleopore filter stained with Irgalan Black. Vacuum pressures did not exceed 10 cm of Hg. The filter was placed on a slide and a drop of immersion oil was placed above the filter and covered with a cover glass (Figure 3).

A Zeiss epifluorescence inverted microscope equipped with a 100 watt mercury bulb and two filter sets (Zeiss 365 excitation filter, 395 dichromatic mirror, 420 barrier filter and Zeiss 450-490 excitation filter, 510 dichromatic mirror, 520 barrier filter), was used for enumeration of picoplankton cells. Picoplankton sized cells that fluoresced a yellow to red color, using the 450-490 excitation filter set, were counted as photoautotrophic cells. For the autotrophic component, a total of 30 microscope fields chosen by using a random fields chart were viewed to determine autotrophic

Figure 3. Staining and counting protocol.



abundance. Careful attention was made in distinquishing autotrophic cells from heterotrophic cells by constantly switching filter sets. Using the ultra violet filter set (365 nm), heterotrophic cells stained with DAPI fluoresce a bluish-white color and therefore were not counted as autotrophic picoplankton. Two cells that had a complete cross wall between cells were counted as a dividing cell. The total number of dividing cells viewed in 30 randomly chosen microscope fields were counted to determine frequency of dividing cells for the autotrophic component of the picoplankton. FDC was determined by dividing the number of dividing cells per field by the number of total cells per field.

Calculation of growth rate

Changes in autotrophic picoplankton abundance and FDC were plotted over the incubation period each month. A best fit line was calculated for data points that were determined within the exponential growth phase of the incubation period. The calculation of the exponential growth phase for all incubations was based on the maximum frequency of dividing cells. From *in vivo* experiments of picoplankton growth, Waterbury et al. (1986) reported the point of maximum cell division coincided with the midpoint in exponential growth phase of the picoplankton cell cycle. To be consistent in the method of determining the exponential phase of growth for each

incubation, the exponential growth phase was defined as all abundance values of the incubation that occurred from t_o through t_{max+1}. The value of t_{max+1} corresponds to one data point beyond the time where the maximum dividing cells was observed. For those months where the maximum FDC was observed during the final collection of the incubation period, all data points of the incubation were used to calculate the best fit line. The calculation of the best fit line for data points within the exponential growth phase was based on a linear fit model relating $\log_{10}$(abundance) to time by minimizing the sum of the squares of the residuals for the fitted line. The origin of the best fit line for all *in situ* incubations was the picoplankton abundance value at t_o.

Using the best fit line, specific growth rates (μ) of the autotrophic picoplankton component for all incubations were determined from a change in cell numbers over time:

$$\mu = (\log_{10}Z - \log_{10}Z_o)\ 2.303/(t - t_o)$$

where Z and Z_o represent the abundance of picoplankton at the incubation times of t_{max+1} and t_o respectively (Stanier et al. 1979).

Calculation of duration of cell division

Duration of cell division (T_d) was calculated for all incubations using the following formula from McDuff and

Chrisholm (1982) which explains growth of phytoplankton populations:

$$\mu = 1/n(T_d) \quad \Sigma \quad \ln(1 + f_i) \qquad (1)$$

where: μ = the specific growth rate (day^{-1}); n = the number of samples; T_d = duration of cell division; f_i = maximum frequency of dividing cells. Daily growth rates were calculated by multiplying the hourly growth rates of each incubation by the total daylight hours of the particular day of incubation. Daylight hours were obtained using climatological data for Norfolk, Virginia (NOAA, 1988a, 1989a). From data describing the duration of cell division of picoplankton for all 15 months of incubations, a decision was made to calculate a correction factor for FDC for the months of December 1988 and February 1989.

Calculation of FDC correction factor

A correction factor was needed for December 1988 and February 1989 to make the relationship between FDC and μ a more practical tool over a wider range of water temperatures that would include those common in winter. The colder water temperatures are known to affect the duration of cell division in the *Synechococcus* sp. cell cycle (Campbell and Carpenter, 1986). This correction factor for December 1988 and February 1989 was calculated using the equation from McDuff and Chrisholm (1982). Knowing the growth rate of picoplankton

for December 1988 and February 1989 (calculated from *in situ* incubations), the equation was solved for f_i, where the average time of cell division calculated from the other 13 incubations of this study was used for T_d. A correction factor for December 1988 and February 1989 was calculated from the following equation:

$$f_i = FDC - [C \times FDC]$$

where f_i = the corrected frequency of dividing cells calculated using equation 1; FDC = the original maximum frequency of dividing cells value calculated from *in situ* incubations for December 1988 and February 1989; C = the correction factor. Both correction factors were averaged and used to correct FDC values found in December 1988 and February 1989. For those water samples where the temperature is less than 9.00 °C, the following correction factor should be used:

$$FDC_{cor} = FDC \times 23\%$$

where FDC_{cor} = the corrected FDC value; FDC = the frequency of dividing cells enumerated in water sample.

Relationship between FDC and μ

After a corrected FDC value for December 1988 and February 1989 was calculated, linear regression was performed on the maximum FDC values (independent variable) and μ values (dependent variable) observed for all 15 incubations of this

study. The regression equation calculated from this statistical procedure expressed the relationship between frequency of dividing cells and growth rate of autotrophic picoplankton in the lower Chesapeake Bay.

Sodium ^{14}C-bicarbonate validation

To check the validity of using the regression equation to estimate autotrophic picoplankton growth rates and productivity in the lower Chesapeake Bay, a blind test was run comparing FDC technique to sodium ^{14}C-bicarbonate analysis for measuring picoplankton productivity. Using the same parcel of water for each procedure, an estimation of productivity was calculated using both sodium ^{14}C-bicarbonate and frequency of dividing cells techniques. This validation procedure took place from July 1989 through October 1989 and again in January 1990.

Sodium ^{14}C-bicarbonate procedure

A one liter water sample from the lower Chesapeake Bay was collected in a one liter plastic bottle, immediately placed on ice and brought back to the Old Dominion Phytoplankton Laboratory for analysis. A 350 ml subsample was filtered through a 2.0 μm Nucleopore filter using pressures no greater than 10 cm of Hg to separate the picoplankton component from larger phytoplankton forms. The filtering process involved filtering the water sample in small fractions

(approximately 25 ml) to avoid clogging the filter; that would inhibit the passage of picoplankton sized cells. Three 100 ml subsamples of the picoplankton sample were placed in three glass incubation bottles and allowed to acclimate for 30 minutes in an incubation apparatus equipped with Cool-White fluorescent lighting and a flowing water bath simulating *in situ* conditions of temperature and light intensity. In addition, three 100 ml subsamples of the original non-fractionated water sample were placed in three glass incubation bottles to acclimate for 30 minutes in the same incubation apparatus. By calculating the amount of productivity that occurred in the sample that had not been fractionated, a better understanding of the contribution of picoplankton productivity to the total amount of phytoplankton productivity was obtained.

To calculate the concentration of stock sodium ^{14}C-bicarbonate used in this portion of the study, a 50 μl aliquot of sodium ^{14}C-bicarbonate was placed in a 7 ml aqueous scintillation cocktail solution. Following phytoplankton and picoplankton acclimation, sodium ^{14}C-bicarbonate (5 μCi in 50 μl) was placed in each of the six incubation bottles. A randomly chosen bottle from the total phytoplankton and picoplankton bottle sets was selected and sacrificed as a time zero sample. A 10 or 15 ml subsample of each bottle was filtered to a 0.2 μm Nucleopore filter to estimate the initial amount of sodium ^{14}C-bicarbonate added to the bottles for a

control. The Millipore chimney apparatus used for filtration was rinsed using two aliquots of filter sterilized seawater. The filter was acid burned using concentrated HCl and placed in a 7 ml non-aqueous scintillation cocktail solution. The rest of the bottles were allowed to incubate for approximately one hour. Starting times of incubation with sodium ^{14}C-bicarbonate were recorded for each bottle.

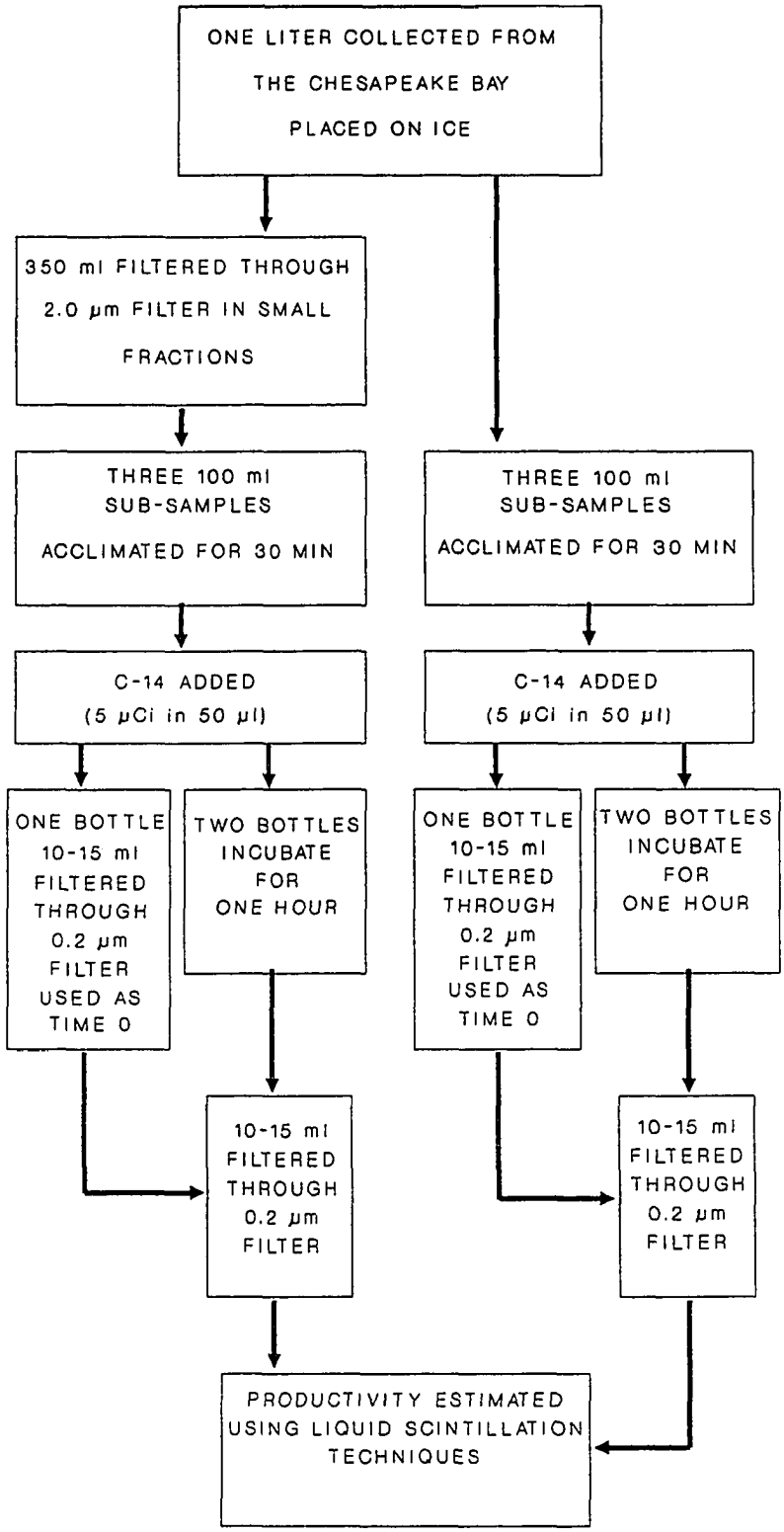
After the incubation period, a 10 or 15 ml subsample from each bottle was filtered on a 0.2 μm Nucleopore filter. The Millipore chimney was rinsed twice using filter sterilized seawater. The official end time of the incubation period occurred when the last rinse passed through the 0.2 μm Nucleopore filter. End times of incubations were recorded for calculating the productivity with this technique. Nucleopore filters were acid burned using concentrated HCl and placed in a 7 ml non-aqueous scintillation cocktail solution (Figure 4).

All cocktail solutions were analyzed using a Beckman LS 1701 liquid scintillation counter. Hourly carbon fixation rates were calculated for replicate subsamples of both picoplankton and total phytoplankton samples using the following formula from Strickland and Parsons (1972):

$$\text{mgC}\cdot\text{m}^{-3}\cdot\text{h}^{-1} = [(dpm1-dpm0)\times(100/V) \times 1.05 \times CA/(Rs \times t1)$$

where $dpm1$ = dpm of replicate subsample; $dpm0$ = dpm of time zero subsample; V = volume of subsample filtered; CA = carbonate alkalinity in mgC m^{-3} (values calculated following

Figure 4. Sodium ^{14}C-bicarbonate protocol.



procedures outlined by Strickland and Parsons (1972)); *Rs* = total ^{14}C dpm in 100 ml sample; *tI* = incubation time for replicate subsample. Picoplankton contribution to total phytoplankton productivity was calculated by dividing the picoplankton productivity value obtained using the sodium ^{14}C-bicarbonate method by the total amount of phytoplankton productivity observed for five months.

Frequency of dividing cells technique

From the regression equation of FDC and μ, an estimate of picoplankton productivity was calculated based on the frequency of dividing cells and total abundance of picoplankton in a water sample. A 125 ml subsample from the original parcel of water collected for the comparison test was fixed using glutaraldehyde (1% final concentration). A 2 to 10 ml subsample was filtered on to a 0.2 μm Nucleopore filter and stained with DAPI. Picoplankton abundance and FDC were enumerated (see enumeration procedures). By substituting the FDC data observed from the water sample into the regression equation and relating FDC and μ previously calculated from this study, a value for the autotrophic picoplankton growth rate was obtained. To estimate the amount of cell growth over a one hour period, total picoplankton abundance was multiplied by the growth rate calculated from the regression equation. A conversion to carbon biomass was made by multiplying the

growth of cells per hour by a value representing the cellular carbon content of one picoplankton cell. A value of 115 fgC per cell was used to represent the cellular carbon of a picoplankton cell (Ray et al., 1989).

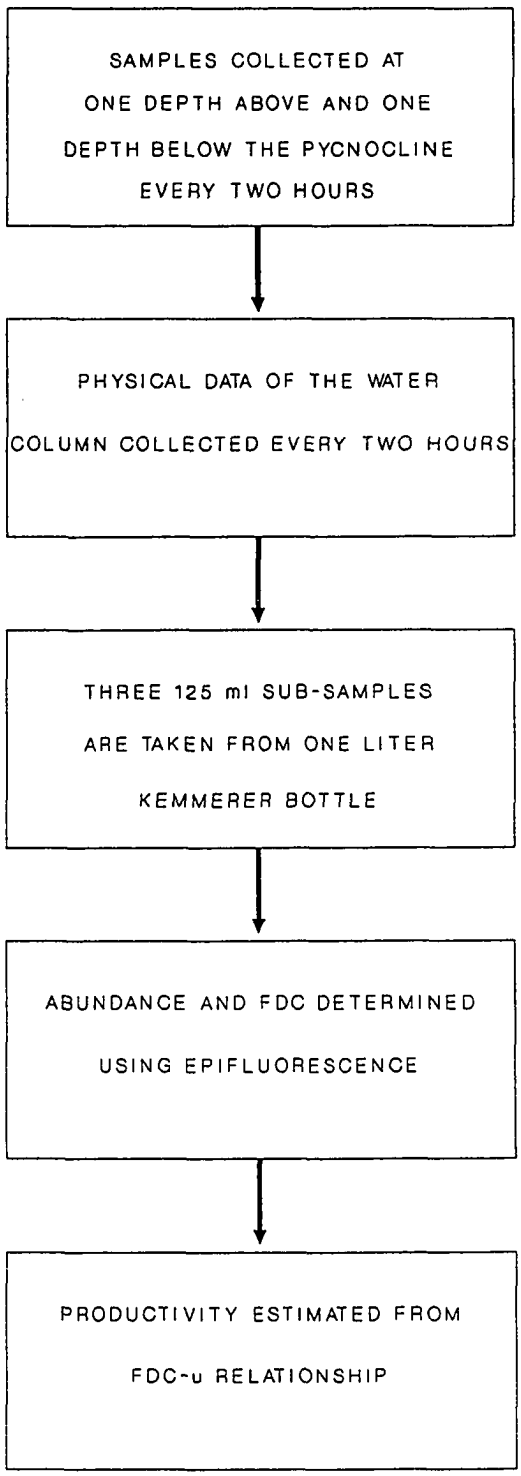
A correlation analysis was run comparing the productivity values calculated using the FDC and the sodium C^{14}-bicarbonate techniques. From this correlation, the validity of the FDC technique to measure picoplankton productivity was evaluated.

High frequency sampling

By sampling the picoplankton on a high frequency time scale (i.e., 2 hr. intervals), more distinct patterns of productivity and abundance in relation to various physical parameters of the water column were obtained.

From the same fishing pier location of the south island, two diel studies were carried out: one in late summer (August 1988), one in winter (January 1989). Samples were collected every two hours over a 24 hour period, one meter below the surface and one meter from the bottom. This collection method was used to sample the picoplankton component above and below the pycnocline during the course of this study (Figure 5). Three replicate samples were collected at the two sampling depths using a one liter Kemmerer bottle. Samples were fixed in 125 ml. Nalgene plastic bottles with glutaraldehyde (1% final concentration). All samples were filtered and stained using DAPI for calculating abundance and FDC values (see

Figure 5. High frequency sampling protocol.



enumeration procedures).

In addition, at two hour intervals, secchi depth, temperature, salinity and conductivity readings were taken at each meter of depth using an inductive salinometer, temperature probe and secchi disk. Pycnocline/thermocline regions of the water column were defined by an abrupt change in salinity, conductivity and temperature over depth. Estimates of productivity for the autotrophic picoplankton component were calculated using the FDC-μ relationship calculated from the previous experiment.

A one-way Model I ANOVA was performed using abundance data for both top and bottom samples to analyze effects of the sampling position on picoplankton abundance. Likewise, a one-way Model I ANOVA was performed using the productivity data for both top and bottom samples of each 24 hour study. This test was performed to analyze the effects that sampling position had on picoplankton productivity. For both of these statistical tests, the variable sampling position was used as a treatment. A series of one-way Model I ANOVA tests were performed on top and bottom abundance and productivity data where the variable time was used as a treatment. These statistical procedures were performed to test whether significant changes in picoplankton abundance and productivity occurred over the 24 hour studies for both top and bottom sampling. Results of these statistical tests were compared to the tidal flux information on the lower Chesapeake Bay area

(NOAA, 1988b, 1989b).

Chapter 4

RESULTS

Control culture

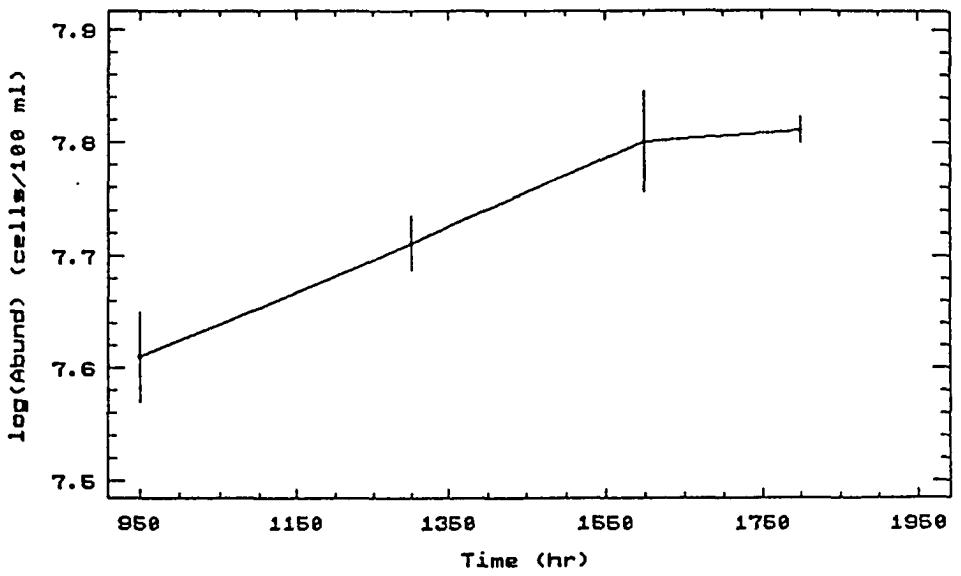
Data describing growth patterns and frequency of dividing cells (FDC) from a pure culture of *Synechococcus* sp. are graphically shown in Figure 6. Results of a one-way Model I ANOVA reveal a significant change in *Synechococcus* abundance and FDC over the 8.5 hour incubation period (time: $P < 0.01$; FDC: $P < 0.01$). There was an approximate 60% increase in the number of *Synechococcus* cells at the end of the incubation period. An increase in the FDC was observed over the first 3.5 hours of incubation with the maximum FDC observed at the second sampling collection (1300 hours). The time of maximum FDC corresponded to the midpoint of *Synechococcus* growth. There was a noticeable decrease in the FDC observed from the 1300 hour sampling period through the remaining hours of incubation.

FDC - μ relationship

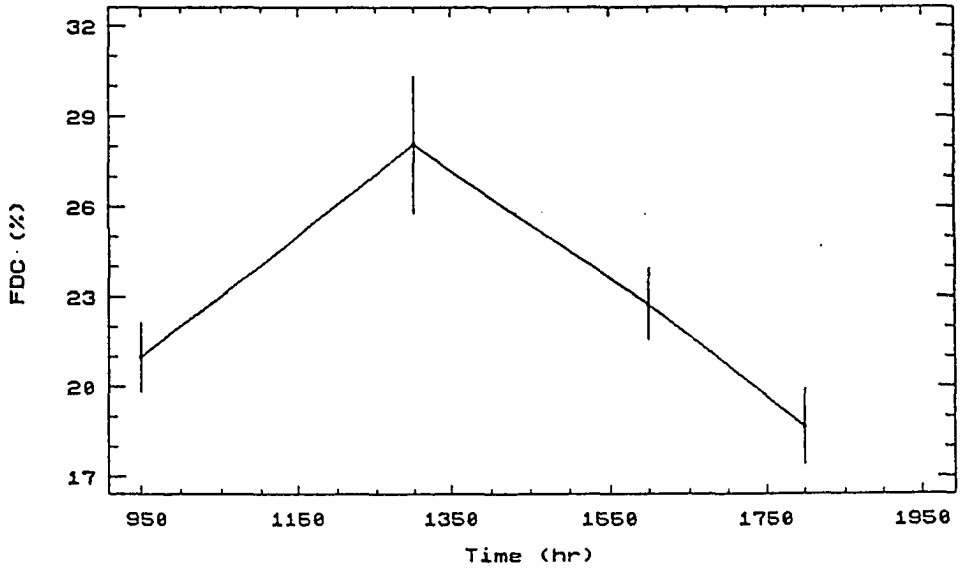
Monthly *in situ* incubation data are presented graphically in Appendix A. A best fit line expressing the change in

Figure 6. Plots of growth (A) and frequency of dividing cells (B) versus time of a pure culture of *Synechococcus* sp..

A



B



growth during the calculated exponential phase of incubation and the point at which the maximum number of dividing cells were observed are plotted for each month. A summary of the maximum frequency of dividing cells and growth rates observed for each incubation is given in Table 1. The maximum number of dividing cells varied from 5.05% to 19.35%, with the higher FDC values occurring in the summer months. Specific growth rates calculated from each incubation varied from 0.23 day^{-1} to 1.10 day^{-1}, with higher growth rates common for the summer period.

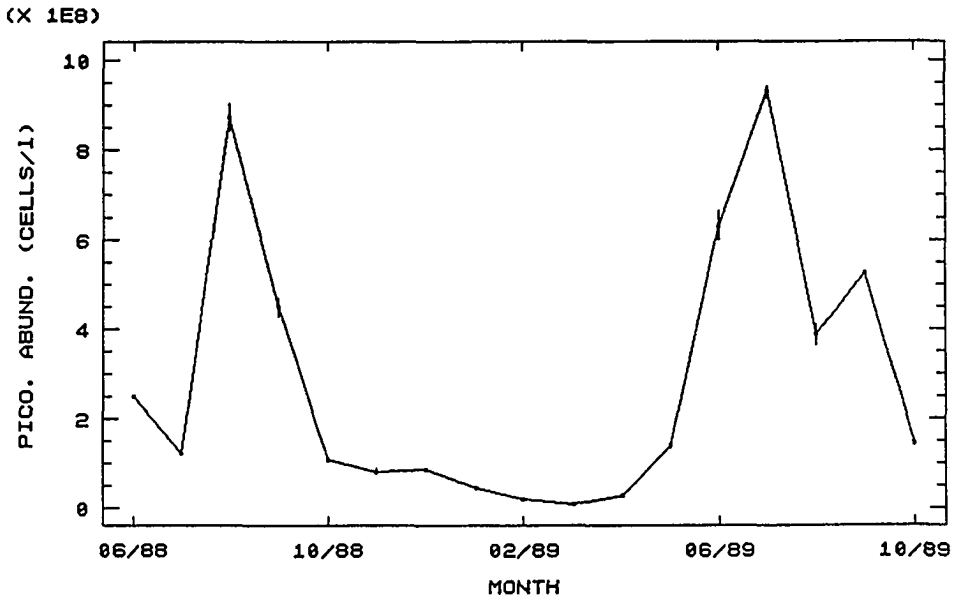
Using the abundance values at t_0 for each *in situ* incubation and top abundance values observed at t_1 for both August 1988 and January 1989 diel studies, seasonal patterns of abundance were recognized. Because *in situ* samples for each incubation were taken from a composite sample of the water column, these abundance values represent average abundance of autotrophic picoplankton for the entire water column. Autotrophic picoplankton abundance ranged from 7.36×10^6 cells/l to 9.28×10^8 cells/l. Lowest abundance was in winter; highest abundance in summer (Figure 7). Similar productivity patterns were observed over the 15 month study. Productivity values ranged from 0.055×10^{-6} μgC/l/hr to 6.30×10^{-6} μgC/l/hr. Lowest productivity was in winter, with highest productivity in summer (Figure 7).

Table 1. Growth rates and maximum FDC values for 15 *in situ* incubations. Maximum FDC values in parentheses indicate corrected FDC values.

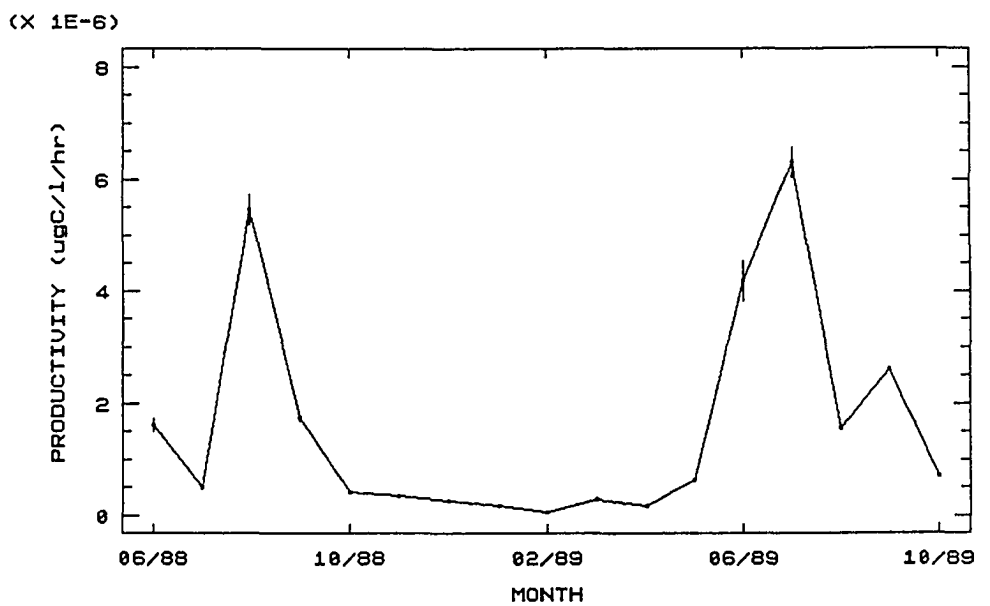
MONTH	GROWTH RATE (hr^{-1})	GROWTH RATE (day^{-1})	MAX. FDC(%)
June 1988	7.46×10^{-2}	1.10	15.81
July 1988	4.81×10^{-2}	0.68	9.80
September 1988	4.65×10^{-2}	0.58	5.99
October 1988	3.94×10^{-2}	0.47	5.05
November 1988	4.47×10^{-2}	0.45	7.22
December 1988	2.39×10^{-2}	0.23	7.05 (1.62)
February 1989	2.58×10^{-2}	0.28	7.21 (1.66)
March 1989	4.35×10^{-2}	0.54	9.42
April 1989	5.94×10^{-2}	0.80	13.11
May 1989	4.47×10^{-2}	0.64	10.17
June 1989	5.85×10^{-2}	0.85	16.28
July 1989	6.63×10^{-2}	0.98	19.35
August 1989	4.26×10^{-2}	0.56	10.62
September 1989	4.35×10^{-2}	0.54	11.80
October 1989	5.14×10^{-2}	0.57	12.36

Figure 7. Seasonal patterns of abundance (A) and productivity (B) for autotrophic picoplankton over the 15 month incubation study. Data points (excluding August 1988 and January 1989) indicate average values over entire water column. Error bars (excluding August 1988 and January 1989) indicate standard error of two replicate samples.

A



B



Calculation of duration of cell division

The FDC technique depends in part on the duration of cell division (T_d) of the picoplankton cells. Estimates for T_d of the picoplankton component for each incubation are given in Table 2. The maximum T_d value took place in December 1988 where water temperature was 6.00 °C. In February 1989, T_d was calculated to be 7.66 hours and water temperature of incubation was 4.62°C. December 1988 and February 1989 represent the highest T_d values observed over the 15 month study. These same two months had the lowest water temperatures observed over the 15 month study in which incubations were performed. The average T_d value of all months, excluding December 1988 and February 1989, was calculated to be 3.81 hours.

Correction of FDC values

Corrected maximum FDC values for December 1988 and February 1989 are presented in Table 1. After correcting for the length of time for cell division during the colder water temperature incubations, the maximum FDC value for December 1988 was reduced from 7.05% to 1.62%, where the maximum FDC value for February 1989 was reduced from 7.21% to 1.66%.

Table 2. Time of picoplankton cell division (T_d) and average water temperature for 15 *in situ* incubations.

MONTH	AVERAGE WATER TEMPERATURE (^{o}C)	T_d (hrs)
June 1988	22.42	2.56
July 1988	25.50	3.53
September 1988	22.58	3.36
October 1988	15.88	3.81
November 1988	13.50	4.71
December 1988	6.00	9.09
February 1989	4.62	7.66
March 1989	9.92	4.34
April 1989	14.29	3.32
May 1989	18.42	3.79
June 1989	25.17	3.35
July 1989	26.00	3.09
August 1989	25.92	4.41
September 1989	26.25	4.68
October 1989	20.50	4.52

Relationship between FDC and μ

Regression analysis relating FDC values over the 15 month study (including the corrected FDC values for December 1988 and February 1989) and μ revealed 81.35% of the variation in μ can be explained by the regression equation of:

$$\mu = 2.37 \times 10^{-3}(FDC) + 0.024$$

where μ equals the growth rate (hr^{-1}); *FDC* is the maximum FDC value observed in natural picoplankton populations (Figure 8).

Comparison of sodium ^{14}C-bicarbonate and FDC techniques

Picoplankton productivity values obtained using the sodium ^{14}C-bicarbonate technique are shown in Table 3. The highest average amount of carbon fixed by the picoplankton occurred in July 1989 (6.58 μgC/l/hr) with the lowest average amount of carbon being fixed in January 1990 (0.109 μgC/l/hr). Picoplankton productivity values obtained using the FDC technique are also shown in Table 3. The highest average amount of carbon fixed by the picoplankton occurred in July 1989 (6.30 μgC/l/hr), with the lowest average amount of carbon being fixed in January 1990 (0.049 μgC/l/hr). Correlation analysis comparing the two techniques used to estimate picoplankton productivity is shown in Figure 9. A positive correlation coefficient of 0.977 was calculated for the two methods of measuring picoplankton productivity with a slope of 0.982.

Figure 8. Relationship of FDC and μ.

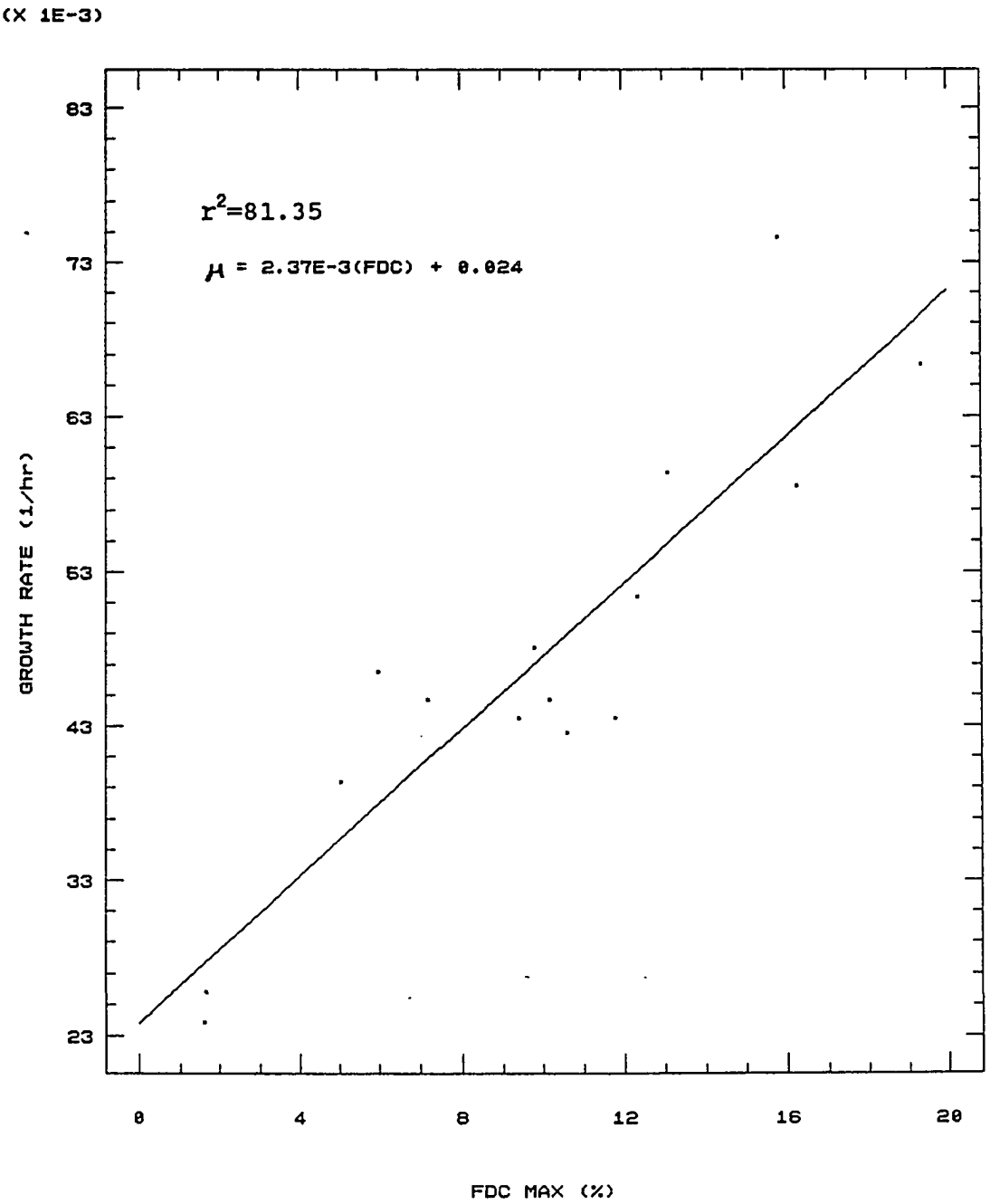
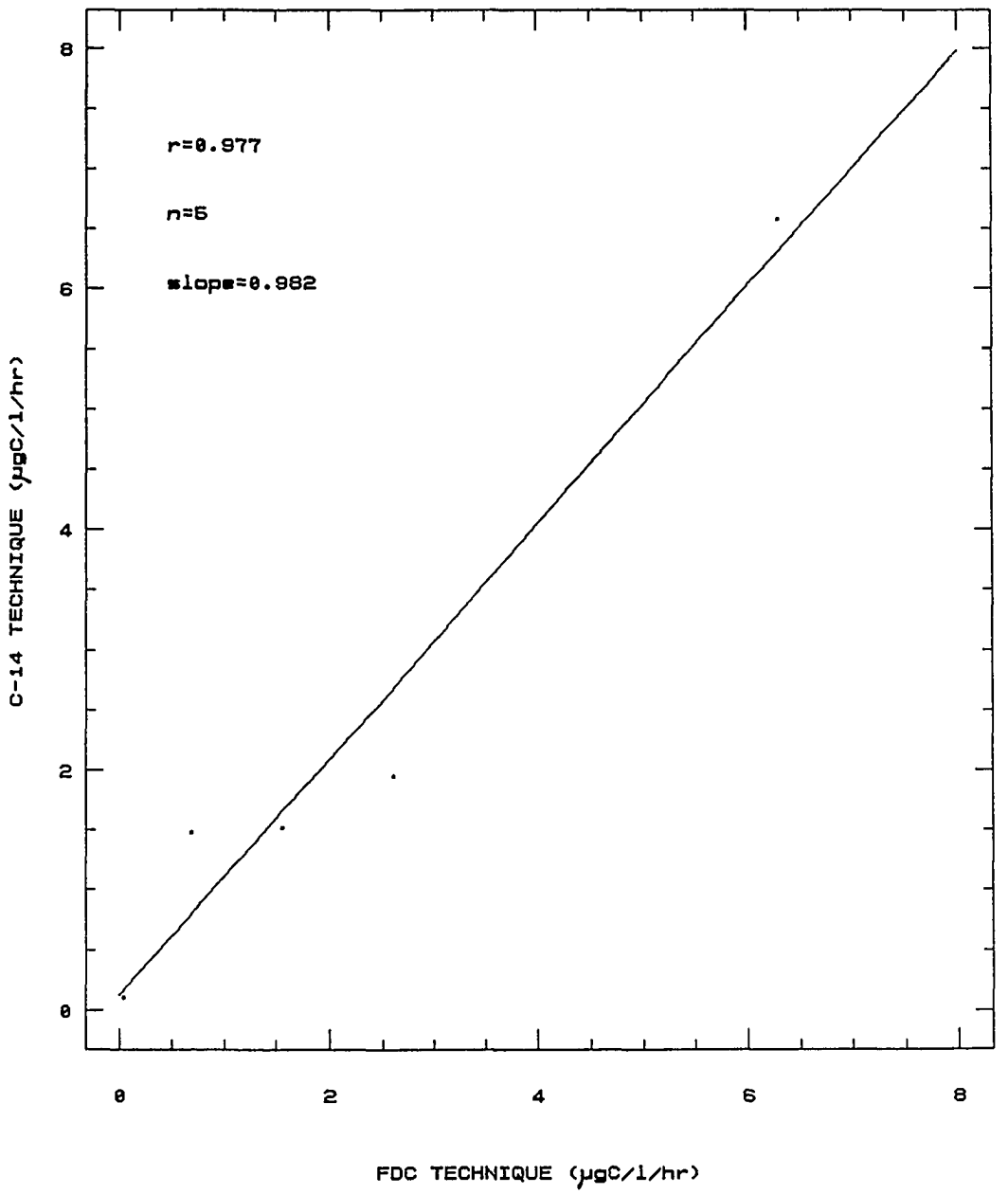


Table 3. Average phototrophic picoplankton productivity values obtained using both sodium ^{14}C-bicarbonate and FDC techniques. Standard error is shown in parentheses. Total productivity values include productivity of cells > 0.2 μm.

MONTH	^{14}C TECHNIQUE (μgC/l/hr)	FDC TECHNIQUE (μgC/l/hr)	TOTAL ^{14}C PROD. (μgC/l/hr)
July 1989	6.58 (0.602)	6.30 (0.270)	12.32 (1.02)
August 1989	1.52 (0.075)	1.56 (0.040)	5.11 (2.28)
September 1989	1.95 (0.142)	2.61 (0.030)	13.67 (0.913)
October 1989	1.48 (0.012)	0.691 (0.015)	19.59 (2.48)
January 1990	0.109 (0.019)	0.049 (0.004)	4.76 (0.119)

Figure 9. Correlation analysis of FDC technique and sodium ^{14}C-bicarbonate technique.

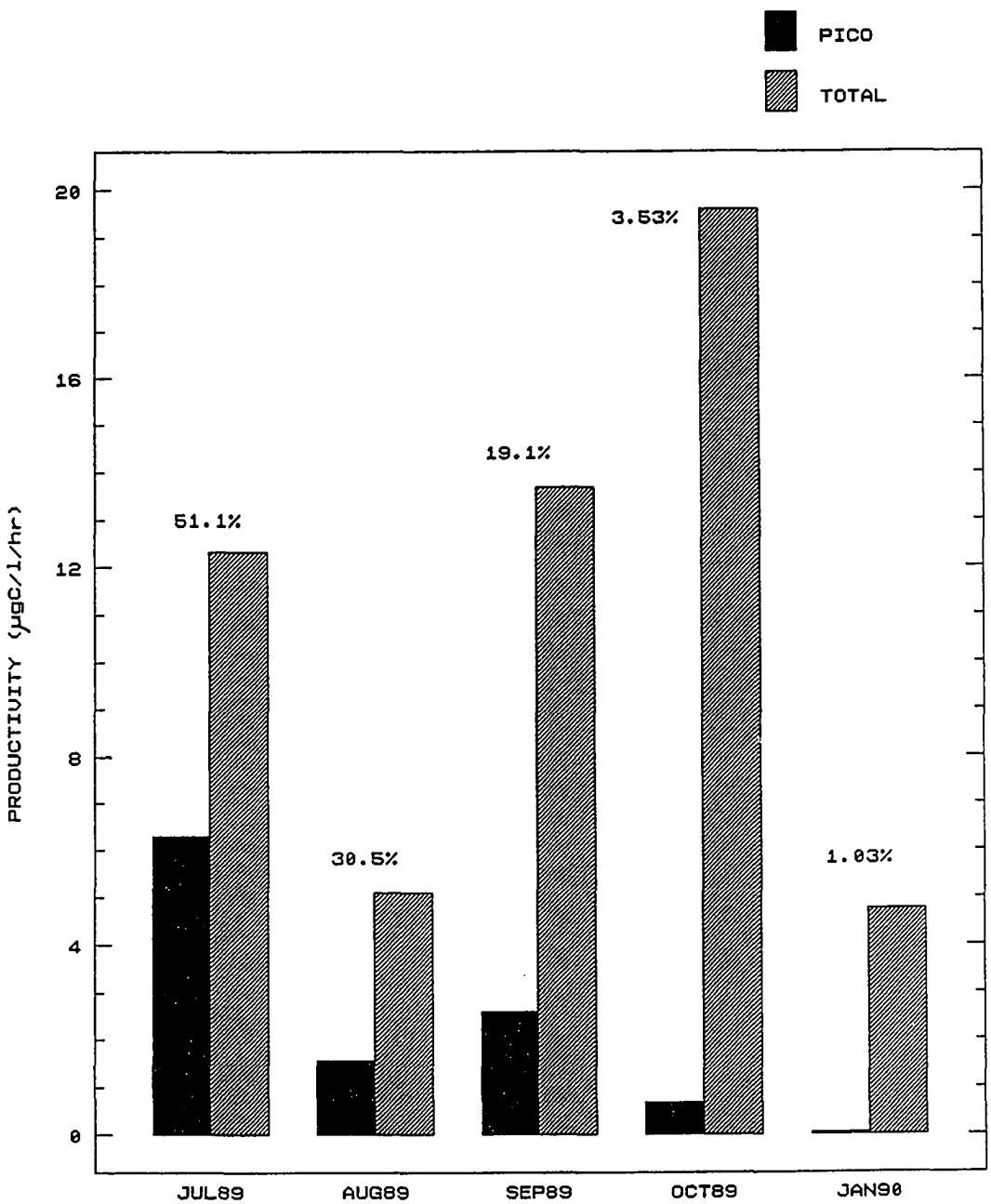


The contribution of picoplankton productivity to total productivity is portrayed in Figure 10 (total productivity values are shown in Table 3). In July 1989, picoplankton were responsible for over half (51.1%) of the total productivity at the collection site. However, picoplankton productivity represented only 1.03% of total productivity in January 1990.

Diel picoplankton abundance and productivity

Physical data (temperature, salinity and conductivity) for diel studies in August 1988 and January 1989 are shown in Appendix B. Temperature readings observed during the August 1988 diel study generally decreased with water depth. Temperature values over the 24 hr study ranged from 26.4°C to 29.24°C at the surface; whereas, temperatures ranged from 17.10°C to 27.08°C near the bottom of the water column. Patterns of conductivity and salinity readings over depth coincided with one another showing a general increase with depth. Conductivity readings varied from 38.39 μmhos/cm to 40.80 μmhos/cm at the surface where conductivity readings near the bottom of the water column ranged from 38.84 μmhos/cm to 41.88 μmhos/cm. Salinity values ranged from 23.40°/°° to 25.82°/°° at the surface and from 23.90°/°° to 31.62°/°° near bottom over the 24 hr study. In August 1988, there was a pycnocline observed that varied in position over time. The pycnocline was observed between two and six meters below the

Figure 10. Contribution of picoplankton productivity to total productivity.

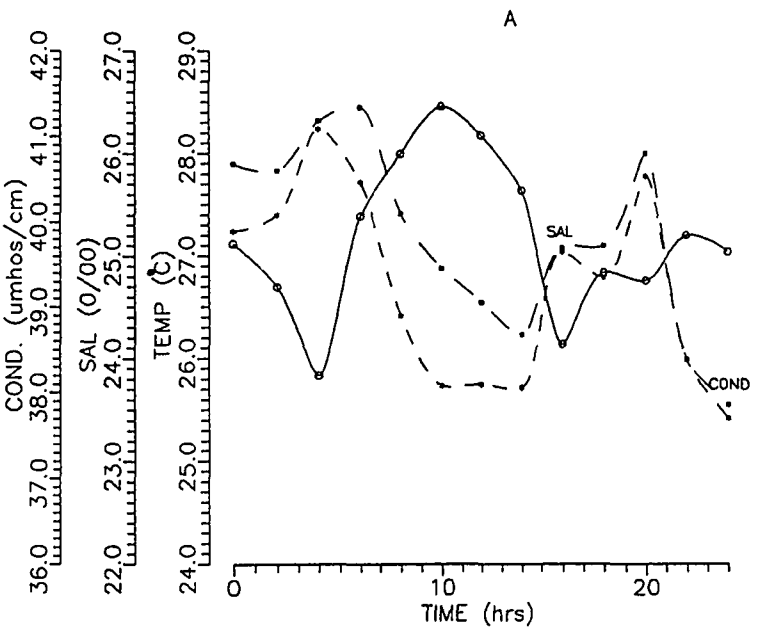


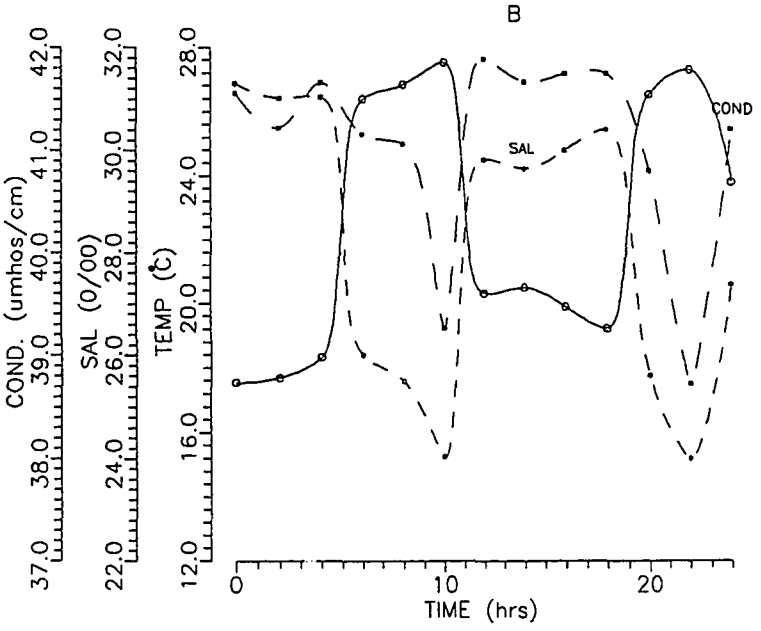
surface over the diel study. There was a break down in the pycnocline region during both ebb tide conditions over the 24 hour period.

Temperature readings for the 24 hr study in January 1989 ranged from 4.9°C to 6.2°C at the surface and 5.2°C to 6.0°C near the bottom. Temperature readings generally increased with depth throughout the diel study. Conductivity and salinity readings also increased with depth. The range of conductivity values for the surface was 22.00 μmhos/cm to 27.10 μmhos/cm and near the bottom from 23.10 μmhos/cm to 30.30 μmhos/cm over the 24 hr study. Salinity readings varied from 21.8°/oo to 27.1°/oo at the surface and 22.9°/oo to 31.0°/oo near the bottom. There was no evidence of a pycnocline during this 24 hr study.

Physical data focusing on the location of sample collection for the August 1988 high frequency study are shown in Figure 11. Salinity values ranged from 23.42°/oo to 26.24 °/oo one meter below the surface of the water and ranged from 24.04°/oo to 31.30°/oo one meter off the bottom over the diel study. Conductivity values ranged from 37.86 μmhos/cm to 41.34 μmhos/cm at the surface and 38.72 μmhos/cm to 41.88 μmhos/cm one meter off the bottom. Water temperature at the surface ranged from 25.84°C to 28.46°C and 17.56°C to 27.52°C at the bottom site during the diel study. Generally, there was an inverse relationship between temperature and salinity at both sites throughout the study. The physical data

Figure 11. Physical data for August 1988 diel study. Measurements taken at depths in water column where high frequency sampling occurred. Graph A represents physical data measured one meter below the surface and graph B represents physical data measured one meter from the bottom at the sampling site.



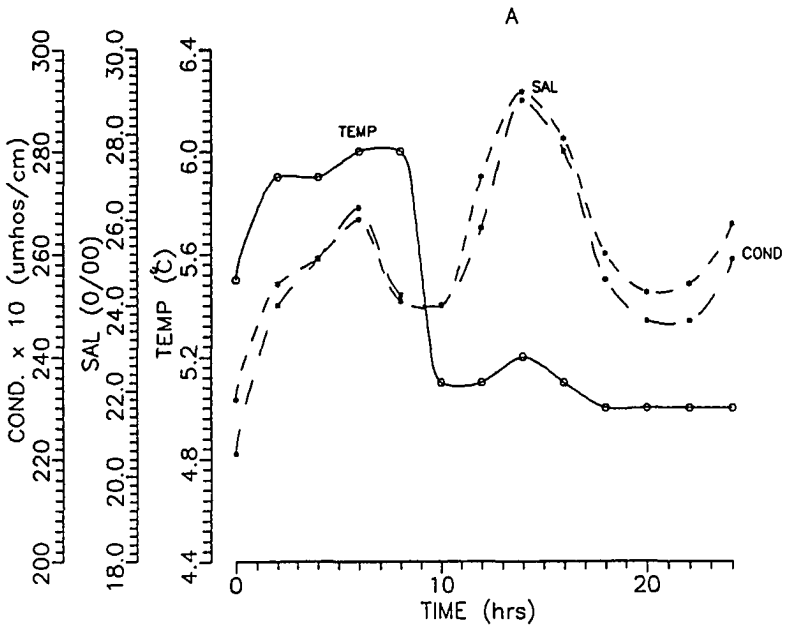


collected for this portion of the study coincided with tidal currents observed for the Chesapeake Bay (NOAA, 1988b).

Physical data isolating the collection sites for the January 1989 high frequency study are illustrated in Figure 12. Salinity values ranged from 21.80$^{o/oo}$ to 27.00 $^{o/oo}$ one meter below the surface and from 22.90$^{o/oo}$ to 31.00$^{o/oo}$ one meter off of the bottom over the diel study. Conductivity values ranged from 22.10 μmhos/cm to 29.00 μmhos/cm at the surface site and 23.10 μmhos/cm to 31.00 μmhos/cm at the sample site one meter off the bottom during the 24 hour study. Water temperature at the upper collection site ranged from 5.00^{o}C to 6.00^{o}C and at the lower site from 5.10^{o}C to 6.00^{o}C over the diel study. There is a direct relationship between temperature and salinity at the upper site throughout the diel study. The physical data collected for this portion of the study coincided with tidal flux data observed for the lower Chesapeake Bay (NOAA, 1989b).

Diel picoplankton abundance at both top and bottom sampling positions for August 1988 is graphically shown in Figure 13. Results of the one-way Model I ANOVA show that sampling position affects picoplankton abundance at the study site ($P < 0.001$). The average picoplankton abundance for the top and bottom sampling positions over the 24 hour study was 8.84×10^8 cells/l and 1.43×10^8 cells/l respectively. Only picoplankton abundance at the lower sampling location was sig-

Figure 12. Physical data for January 1989 diel study. Measurements taken at depths in water column where high frequency sampling occurred. Graph A represents physical data measured one meter below the surface and graph B represents physical data measured one meter from the bottom at the sampling site.



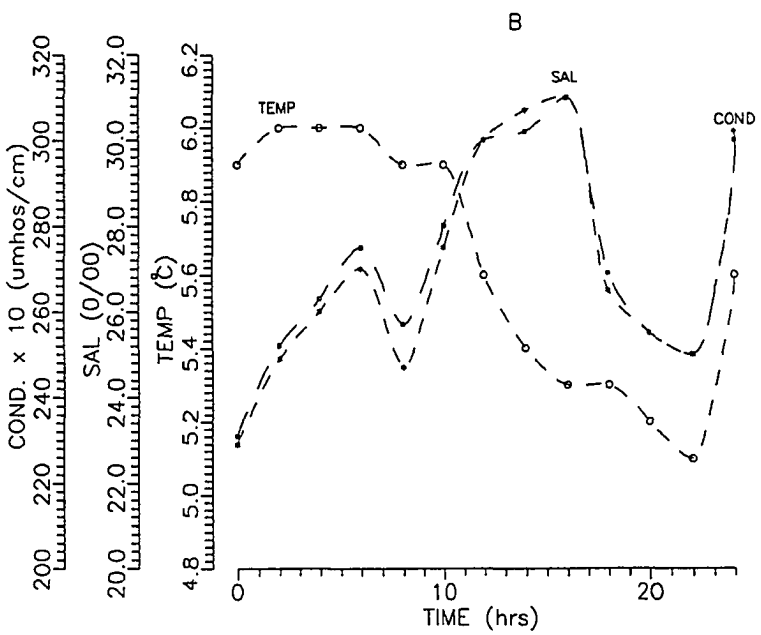
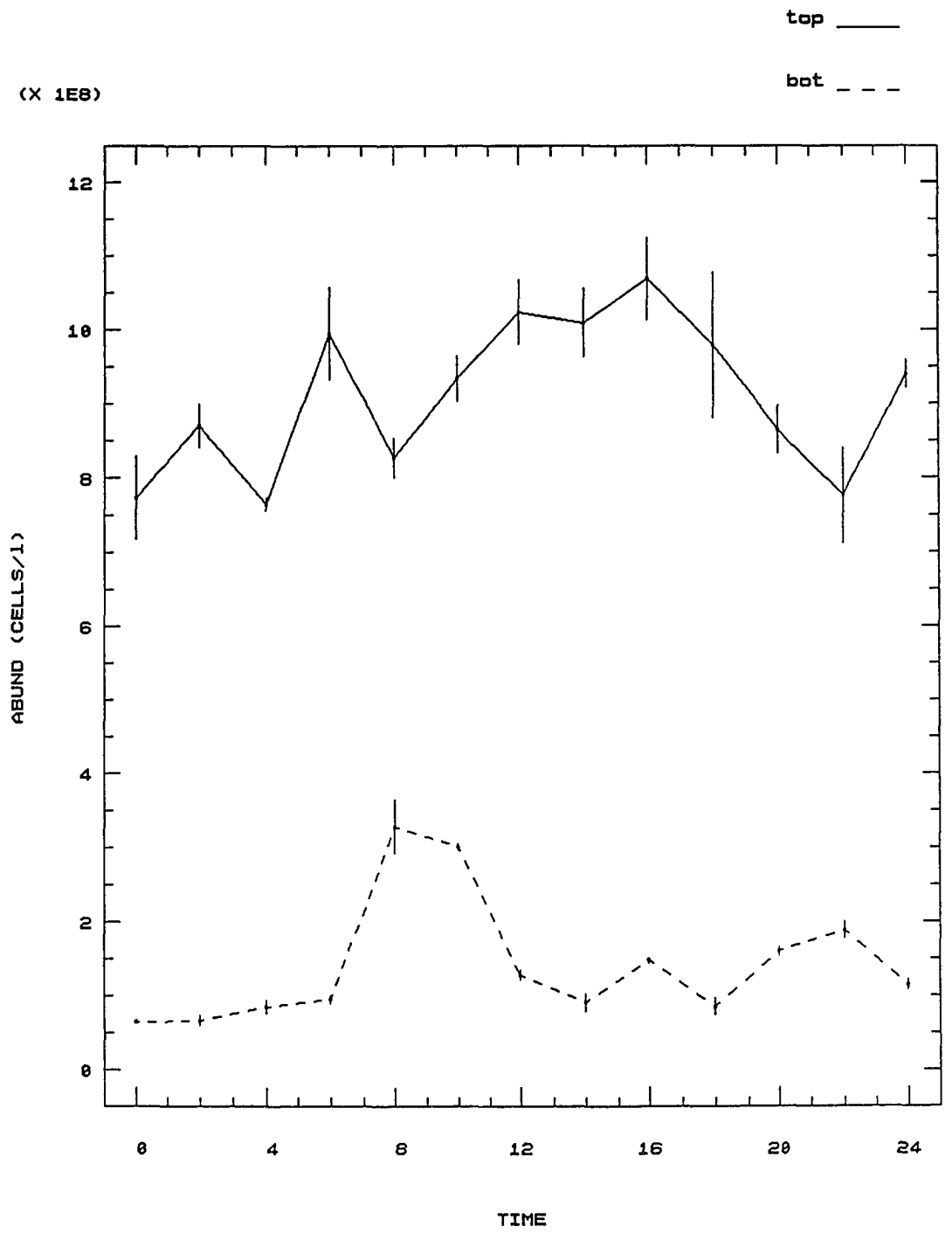


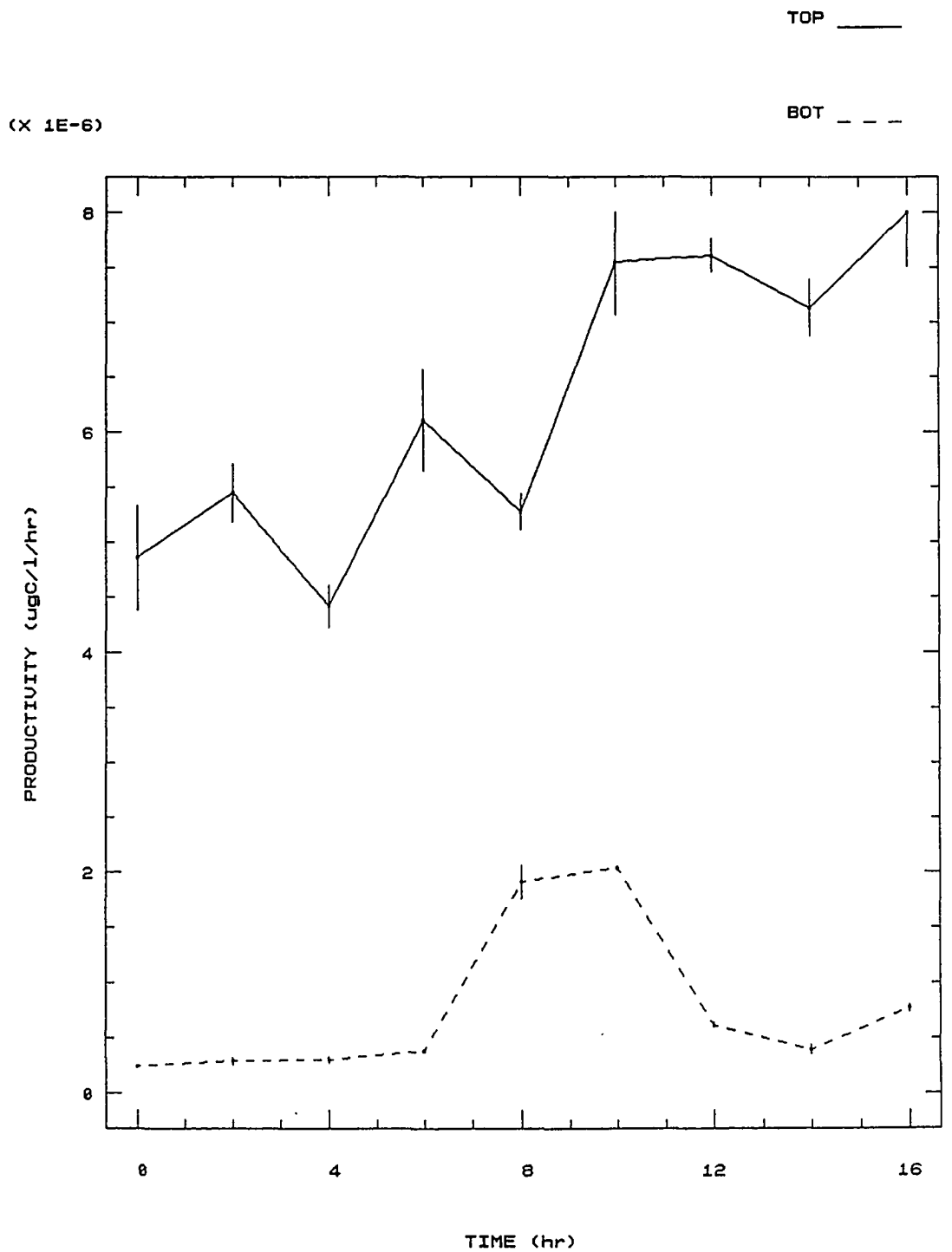
Figure 13. Diel picoplankton abundance at top and bottom sampling positions for August 1988 study. Sampling began at 06:00 hrs (t_0) on August 10, 1988 and ended at 06:00 hrs (t_{24}) on August 11, 1988. Error bars represent standard error values for three replicate samples.



nificantly affected by time over the 24 hour study, with an increase in cell abundance observed between t_3 and t_{10} ($P < 0.001$). Results from Tukey's multiple comparison tests showed picoplankton abundance at t_8 and t_{10} to be markedly different from picoplankton abundance at the other sampling times of the 24 hour study.

The results of diel picoplankton productivity using the FDC technique (at the top and bottom sampling sites) for August 1988 is shown in Figure 14. The FDC values at both top and bottom sampling sites for the diel study in August 1988 are graphically portrayed in Appendix C. Results of the one-way Model I ANOVA showed that sampling position had a significant effect on picoplankton productivity over the 16 hour study ($P < 0.001$). At the top sampling location, time had a significant effect on picoplankton productivity during daylight hours ($P < 0.001$). Results of Tukey's multiple comparison tests revealed picoplankton productivity from t_0 through t_8 was significantly different from picoplankton productivity at t_{10} through t_{16}. The average picoplankton productivity for the top sampling location is 6.27 μgC/l/hr, with lower productivity values observed in the morning and higher productivity values being observed in late evening. Time had a significant effect on picoplankton productivity

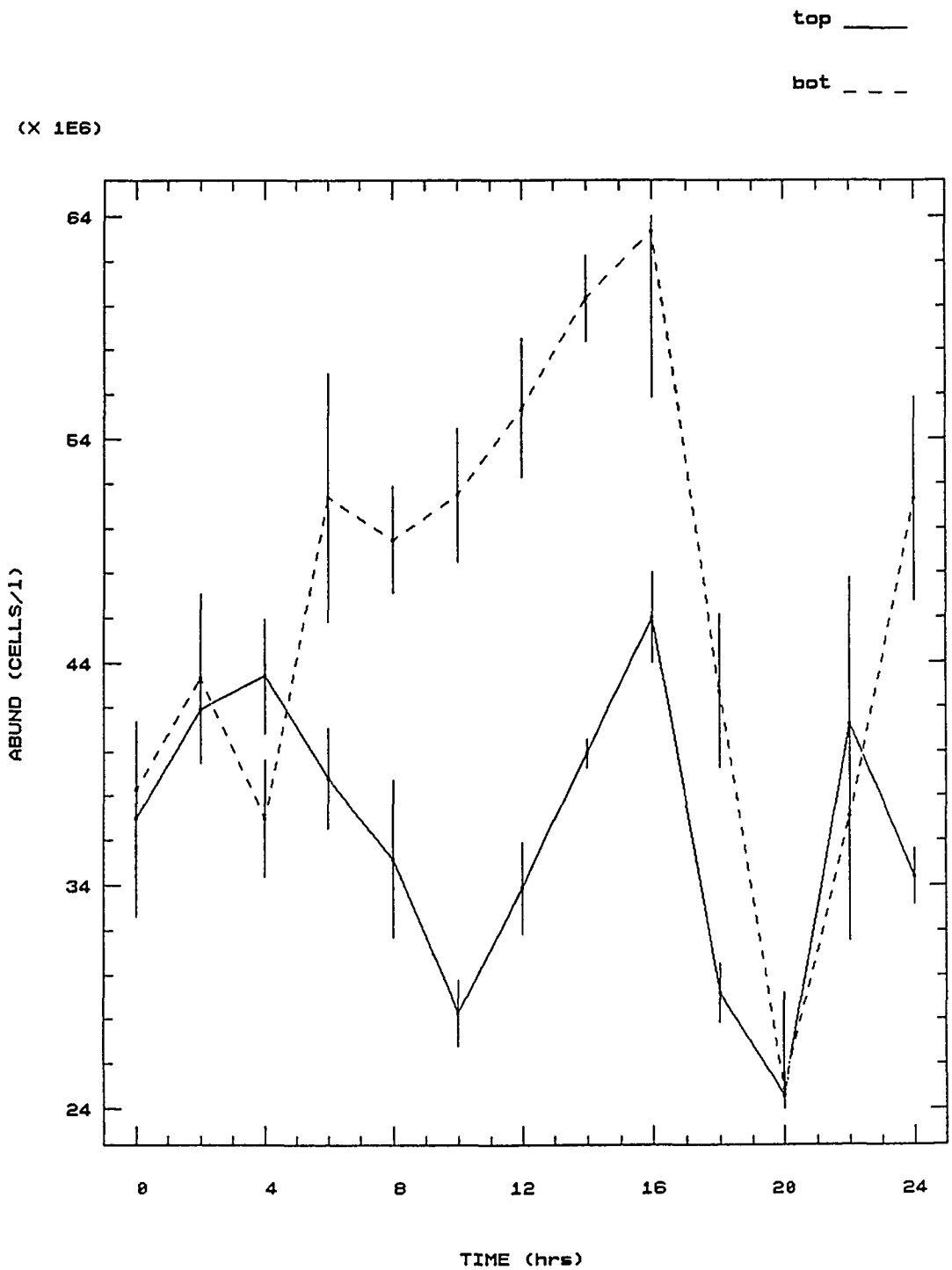
Figure 14. Picoplankton productivity for both top and bottom sampling sites over the daylight hours for the August 1988 diel study using the FDC technique. Error bars represent standard error values for three replicate samples.



over daylight hours at the bottom sampling site ($P < 0.001$). Tukey's multiple comparison testing revealed productivity values at t_8 and t_{10} being significantly different from other productivity values observed throughout the daylight. The average picoplankton productivity for the bottom in August 1988 was 0.77 μgC/l/hr.

Diel picoplankton abundance at both top and bottom sampling positions for January 1989 is graphically shown in Figure 15. Results of the one-way Model I ANOVA show that sampling position significantly affected picoplankton abundance at the study site ($P < 0.001$). The average picoplankton abundance for the top and bottom sampling positions over the 24 hour study was 3.65×10^7 cells/l and 4.66×10^7 cells/l respectively. Picoplankton abundance at the top sampling location was significantly affected by time over the 24 hour study with maximum abundance values observed at t_4 and t_{16} ($P < 0.001$). Tukey's multiple comparisons tests revealed picoplankton abundance at t_4 and t_{16} was significantly different from picoplankton abundance at t_{10} and t_{20}. Picoplankton abundance at the lower sampling location was significantly affected by time over the 24 hour study with a maximum cell abundance observed at t_{16} ($P < 0.001$). Tukey's multiple comparison tests revealed picoplankton abundance at t_{16} being significantly different from picoplankton abundance at t_{20}.

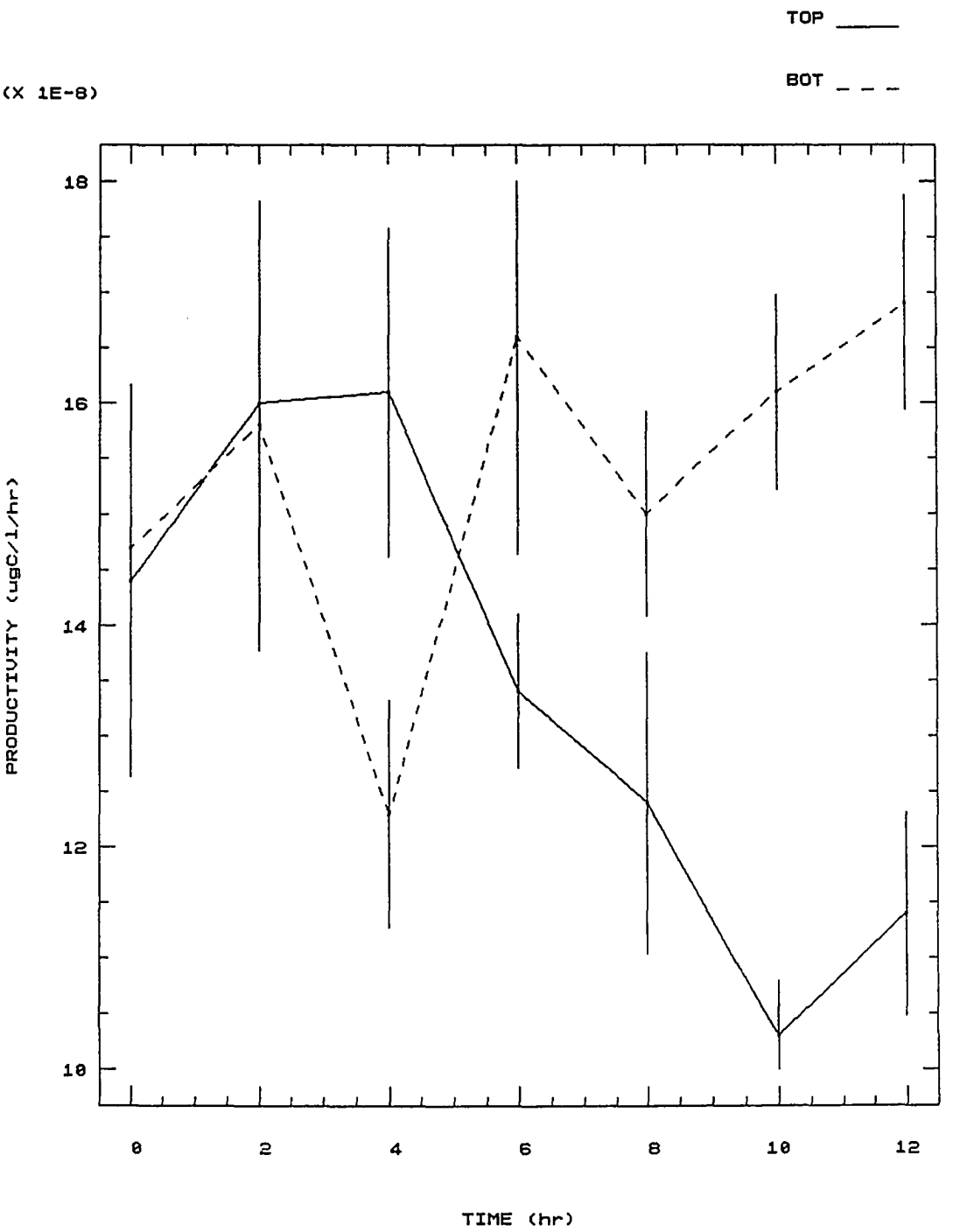
Figure 15. Diel picoplankton abundance at top and bottom sampling positions for January 1989 study. Sampling began at 06:00 hrs (t_0) on January 5, 1989 and ended at 06:00 hrs (t_{24}) on January 6, 1989. Error bars represent standard error values for three replicate samples.



The results of diel picoplankton productivity as determined using the FDC technique at the top and bottom sampling sites for January 1989 are shown in Figure 16. The FDC values at both top and bottom sampling sites for the diel study in January 1989 are graphically portrayed in Appendix C. Results of a one-way Model I ANOVA used to investigate the effects that sampling position had on picoplankton productivity, revealed sampling position as having a significant effect on picoplankton productivity over the 12 hour study ($P < 0.01$). At the top sampling location, time had a significant effect on picoplankton productivity over the daylight hours ($P < 0.05$). Tukey's multiple comparison testing showed picoplankton productivity at t_4 was significantly different from picoplankton productivity at t_{10}. The average picoplankton productivity for the top sampling location was 0.134 μgC/l/hr, with a maximum productivity value at t_4 and a minimum productivity value at t_{10}. The average picoplankton productivity for the bottom site in January 1989 was 0.153 μgC/l/hr, with minimum productivity at t_4 and maximum productivity at t_{12}.

An inverse relationship was observed between picoplankton abundance and salinity during the diel study in August 1988 at the lower sampling site. Because time did not show a significant effect on picoplankton abundance for the top

Figure 16. Picoplankton productivity for both top and bottom sampling sites over the daylight hours for the January 1989 diel study. Error bars represent standard error values for three replicate samples.



sampling location, a comparison of picoplankton abundance with tidal flux was not performed. At the bottom sampling site, picoplankton abundance maxima were observed during low tide conditions (Figure 17). In contrast, during slack flood tide, picoplankton abundance was low relative to picoplankton abundance observed over the 24 hour period.

During the diel study in January 1989, both salinity and picoplankton abundance patterns were in sequence at both sampling locations in the water column. Maximum picoplankton abundance at the top sampling site coincided with maximum salinity values, where during slack ebb tide conditions, picoplankton abundance was low (Figure 18). Similar findings were noted at the lower sampling location as maximum salinity readings coincided with maximum picoplankton abundance values. Picoplankton cell numbers increased with the flooding tide and cell numbers decreased with the ebbing tide (Figure 19).

Figure 17. Comparison between picoplankton abundance (left axis) and salinity (right axis) for bottom sampling site during August 1988 diel study. Tidal information taken from NOAA (1988b) shown on top axis: FT (flood tide), SFT (slack flood tide), ET (ebb tide), SET (slack ebb tide).

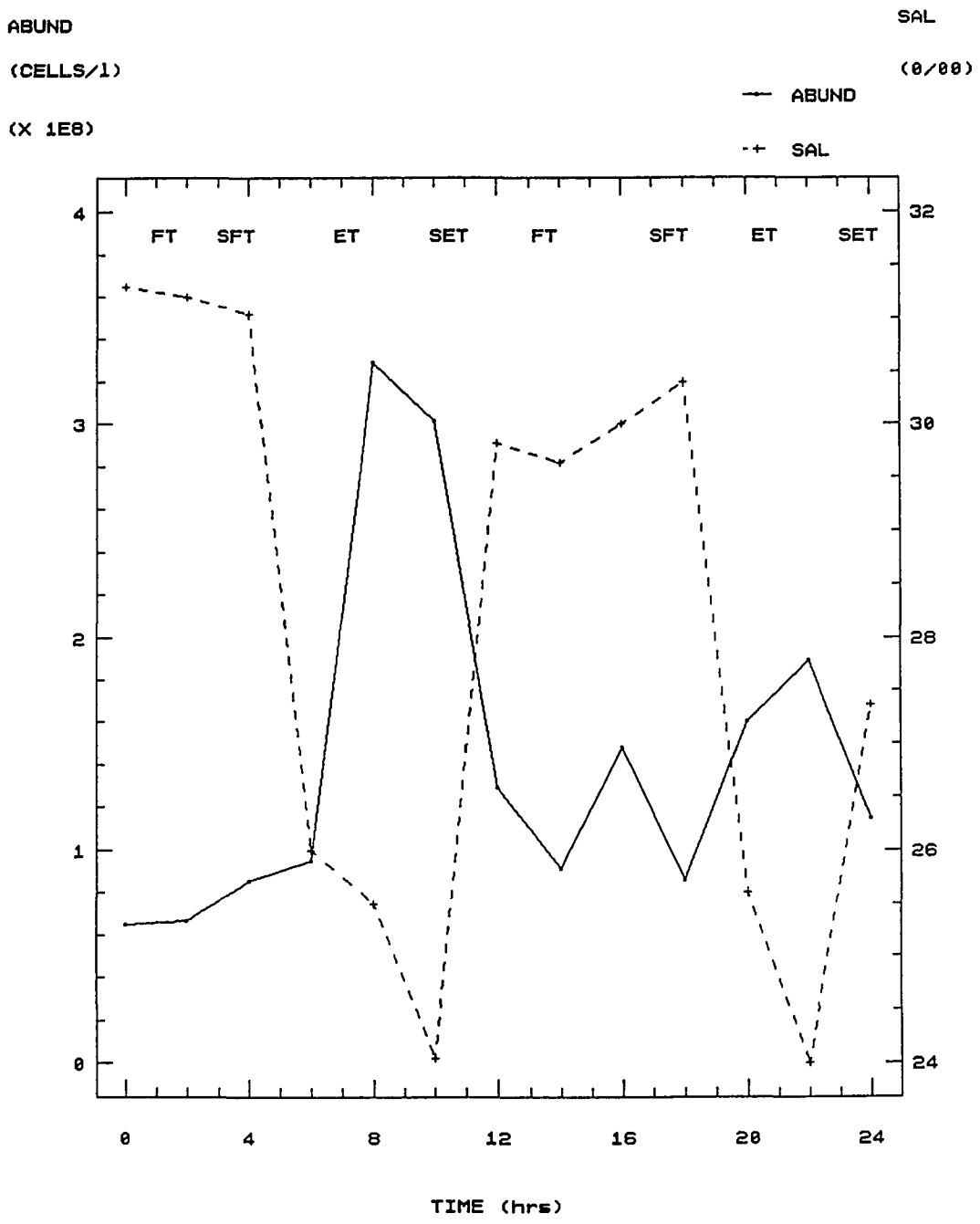


Figure 18. Comparison between picoplankton abundance (left axis) and salinity (right axis) for top sampling site during January 1989 diel study. Tidal information taken from NOAA (1989b) shown on top axis: FT (flood tide), SFT (slack flood tide), ET (ebb tide), SET (slack ebb tide).

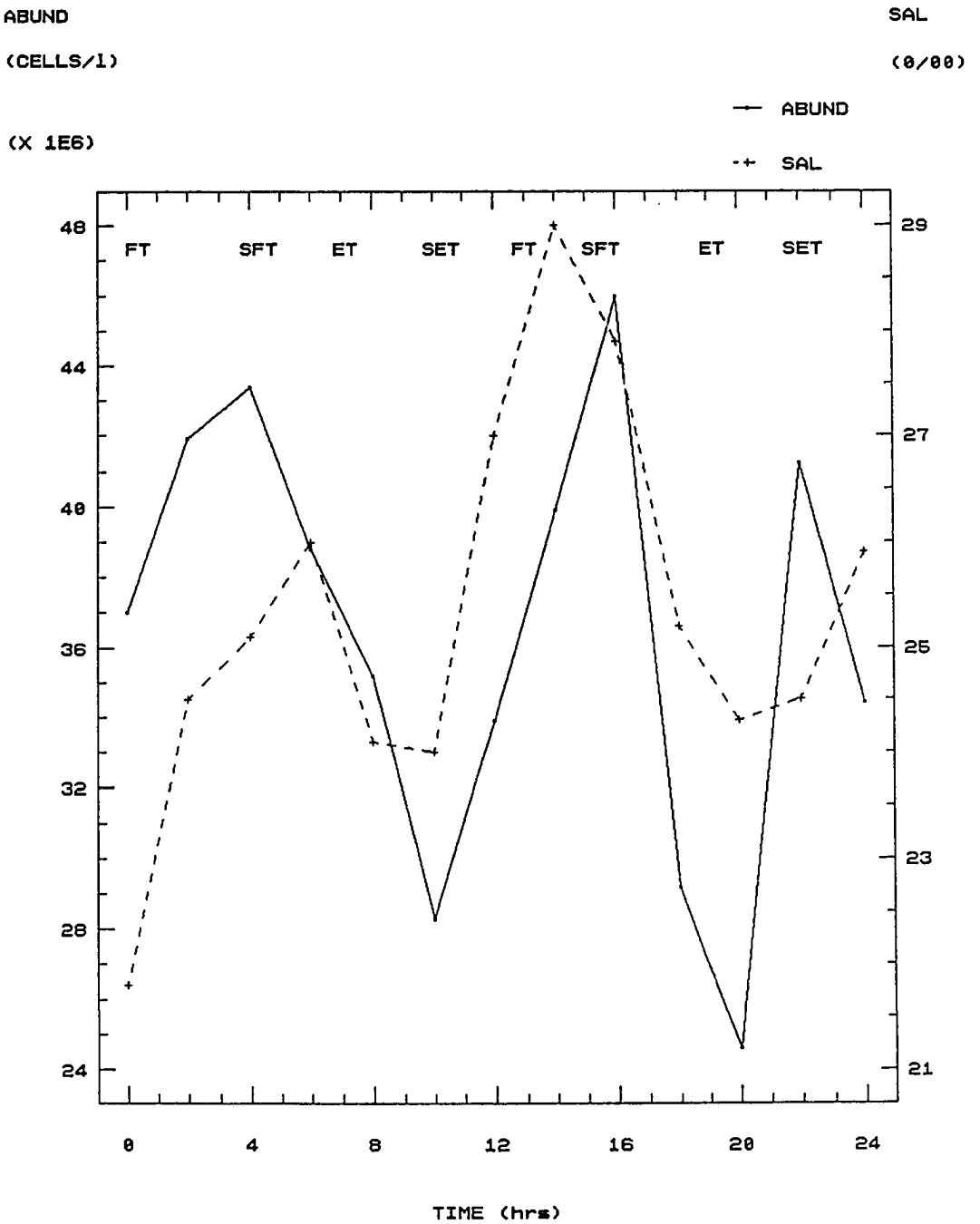
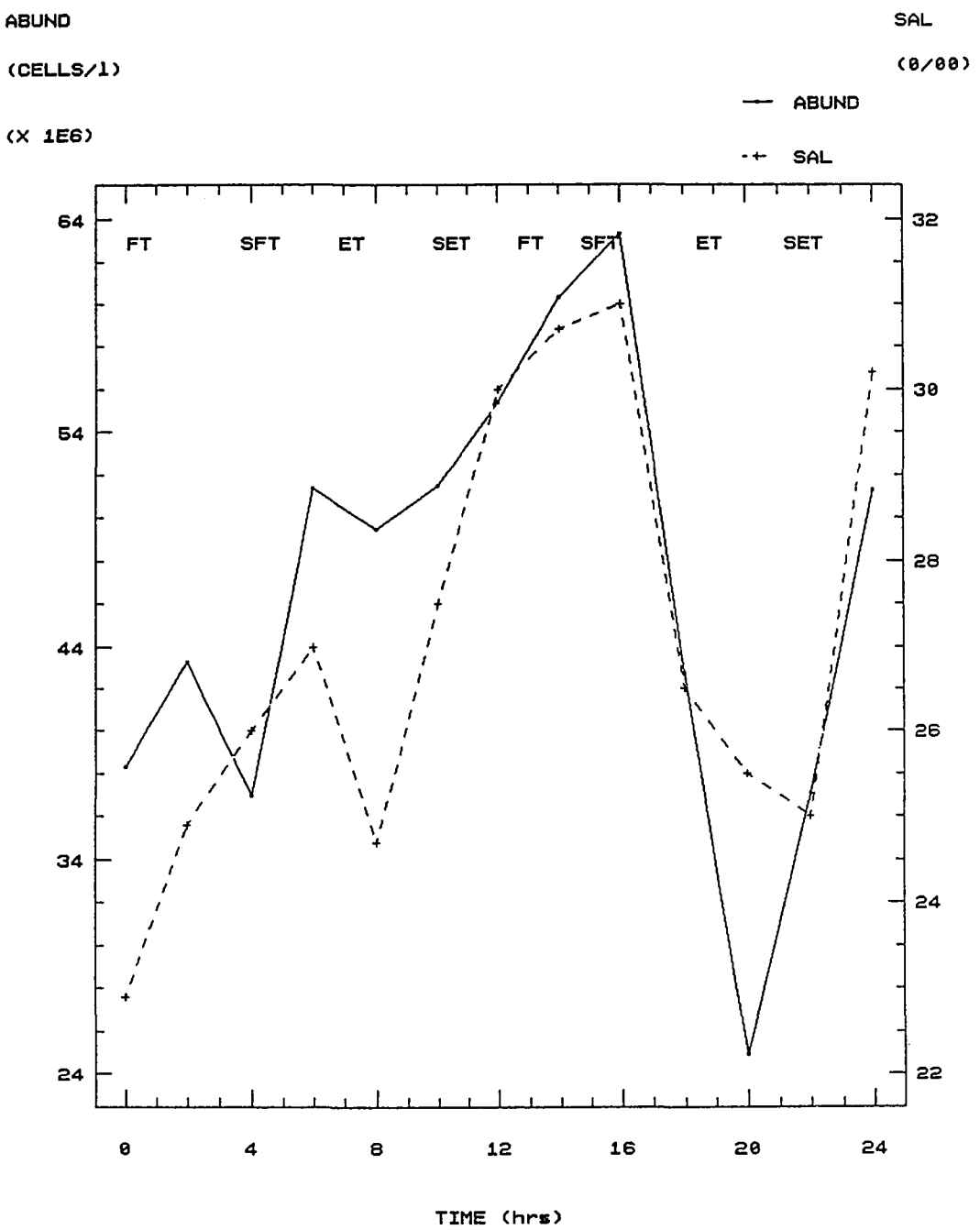


Figure 19. Comparison between picoplankton abundance (left axis) and salinity (right axis) for bottom sampling site during January 1989 diel study. Tidal information taken from NOAA (1989b) shown on top axis: FT (flood tide), SFT (slack flood tide), ET (ebb tide), SET (slack ebb tide).



Chapter 5

DISCUSSION

The frequency of dividing cells (FDC) technique was first developed by Hagström et al. (1979) to measure growth rates of bacterioplankton in aquatic environments. This technique is an alternative method to radioisotope techniques and can ultimately be used to estimate productivity (Hagström et al., 1979; Newell and Christian, 1981; Davis and Sieburth, 1984; Hanson et al., 1983). The FDC technique is based on theoretical and experimental evidence that the number of cells undergoing division is directly related to cell growth rate (Hagström et al., 1979). In exponentially growing cultures, the maximum number of dividing cells theoretically should occur at that point of maximum exponential growth. A preliminary test to verify a unimodal pattern in picoplankton cell division was performed by incubating a pure culture of *Synechococcus* sp. *in vivo*. This organism is a major component of autotrophic picoplankton in the lower Chesapeake Bay (Affronti and Marshall, 1990a, 1990b). Maximum FDC occurred near the mid-point of exponential growth, after which the FDC decreased. These findings are similar to those of Waterbury et al. (1986) and Campbell and Carpenter (1986) whose incubation data

involved both natural picoplankton populations and isolated cultures of *Synechococcus* sp..

To establish the relationship between number of cells undergoing division and bacterial growth, past studies using the FDC technique developed a series of growth incubations under laboratory conditions where temperature, light intensity and nutrient concentration were manipulated to mimic natural conditions. Taking advantage of the unimodal pattern of division in picoplankton, Campbell and Carpenter (1986) employed the FDC technique to estimate autotrophic picoplankton productivity in the Sargasso Sea. Because there was a concern of altering the growth characteristics of picoplankton, growth was not determined using incubation techniques as was used in other FDC experiments on bacterioplankton; rather, picoplankton growth was determined using a mathematical equation developed by McDuff and Chrisholm (1982).

A concern with the use of the FDC technique involves *in vitro* cultures to determine growth rates and their inability to accurately mimic *in situ* growth conditions (Hanson et al., 1983; Newell and Christian, 1981). To minimize this criticism of the FDC method, a decision was made to develop the relationship between FDC and μ by using incubations of natural picoplankton populations typical of estuarine environments *in situ*, rather than use laboratory incubations. In this way,

incubations of picoplankton populations are exposed to natural light and temperature regimes common to the study area. By performing incubations over a 15 month period, a more accurate account of picoplankton seasonal behavior can be assessed when picoplankton cells are exposed to a variety of physical conditions. Most studies using the FDC technique are based on limited temperature and light regimes where these factors are controlled by conditions such as time and length of cruise. Due to the unique ability for easy access to the sampling location at any time during the year, incubation temperatures and light intensities were not restricted and therefore were not a concern in this study. A second criticism of the FDC technique involves interference of growth rates due to grazing pressure (Newell and Christian, 1981; Hanson et al., 1983). To reduce these grazing pressures, *in situ* incubations were performed using diluted picoplankton populations. Even though grazing probably was not completely eliminated, similar productivity values using both FDC and sodium ^{14}C-bicarbonate techniques revealed that grazing was not a significant factor in reducing cell growth in this study. Based on *in situ* picoplankton incubation data, only two of the 15 incubations showed any evidence of the existence of a lag phase in growth (Appendix A). The most likely reason for not noting a lag phase was due to the frequency of sample collections. By sampling at higher frequencies (i.e., every hour),

a lag phase would be more evident. To be consistent in the process of determining picoplankton growth rates, a decision was made to include picoplankton abundance at t_0 as the beginning data point of exponential growth. In using this technique to determine exponential growth, conservative estimates of picoplankton growth rate would be expected in cases where a lag phase was present.

Knowing those factors which affect picoplankton cell division is extremely important if the FDC technique is to be used properly with photoautotrophic picoplankton. Data from this study revealed the duration of picoplankton cell division (T_d) increases in lower water temperatures of incubation. A significant increase in T_d was noted in water temperatures less than 9.00 °C (December 1988: T_d= 9.09 hr, temperature = 6.00 °C; February 1989: T_d= 7.66 hr, temperature = 4.62 °C) where the duration of cell division was two times that of the average T_d for picoplankton cells grown in temperatures greater than 9.00°C. Similar results have been reported by Campbell and Carpenter (1986) using picoplankton typical of oceanic environments as T_d was found to increase in water temperatures less than 15 °C. In water temperatures below 9.00 °C in the lower Chesapeake Bay, a correction factor (FDC_{cor} = FDC × 23%) based on the average duration of cell division in temperatures greater than 9.00 °C should be used

for a better estimate in the relationship between FDC and μ for picoplankton populations. By correcting for the increased duration of cell division in colder water temperatures, the FDC technique can be utilized over a wide range of water temperatures, making the technique more useful year round.

Maximum FDC values of picoplankton obtained for the early summer period (May and June) from *in situ* incubations are similar to those values reported by Waterbury et al. (1986) for *Synechococcus* populations in Buzzards Bay, Massachusetts during a similar period. Higher FDC values observed in the summer months compared to winter can be explained by higher light intensities and incubation temperatures typical of this period. These findings are consistent with data reported by Campbell and Carpenter (1986) where higher FDC values were observed in pure cultures of *Synechococcus* grown at higher temperatures and light intensities. The range of growth rates for this 15 month study was similar to growth rates of phototrophic picoplankton reported by Campbell and Carpenter (1986) at similar incubation temperatures.

Regression analysis relating FDC and μ (including corrected FDC values for December 1988 and February 1989) revealed a reasonable scattering of μ on FDC. A r^2=81.35 provides reasonable confidence in the predictive capacity of the regression line of $\mu = 2.37 \times 10^{-3} (FDC) + 0.024$ in estimating picoplankton growth rates from FDC values in the

lower Chesapeake Bay. In this study, the most labor intensive and time consuming portion of setting up the FDC technique has been accomplished. Picoplankton productivity can easily be determined using direct counting procedures by obtaining the following information: 1) percent of the dividing cells in the population, 2) picoplankton abundance, and 3) the water temperature of the sample to determine if the FDC correction factor should be used. Advantages of this procedure include a rapid method to determine picoplankton productivity without the need for incubating procedures, nor the use of expensive and potentially toxic radioisotope techniques. From these results, it is emphasized that there is no advocation to eliminate or replace the sodium ^{14}C-bicarbonate method as a viable technique to measure picoplankton productivity. Rather, in those situations where time is limited (ie., high frequency studies), radioisotopic techniques are not available or for verification procedures, the FDC technique would be more practical.

As a final verification of the use of the FDC technique to estimate picoplankton productivity, an experiment was performed where picoplankton productivity was estimated using both FDC technique (developed from this study) and standard fractionation methods where picoplankton were incubated using sodium ^{14}C-bicarbonate. The ^{14}C method is the common method for measuring picoplankton productivity in marine systems (Li et al., 1983; Platt et al., 1983; Smith et al., 1985). Even

with this broad application, problems such as bottle effects, trace metal contamination, and sample fixation have been identified as potential sources of error in accurately measuring productivity using this technique (Carpenter and Lively, 1980). The five month study varied from the summer season to the winter season to include a wide range of physical conditions for picoplankton growth. This study also included a sample taken in water temperatures less than 9.00 ^{o}C to verify the correction factor to be used for such cold water conditions. A high correlation coefficient of 0.977 comparing the two methodologies further emphasizes the usefulness and accuracy of the FDC technique as an alternative technique to estimate picoplankton productivity. One limitation to the FDC technique is the lack of consideration for growth in terms of an increase in cell mass. This inaccuracy may ultimately affect productivity rates and most likely explains why the majority of productivity rates using the FDC technique are lower compared to sodium ^{14}C-bicarbonate fractionation. Other factors contributing to lower production values using the FDC technique compared to sodium ^{14}C-bicarbonate technique involves possible uptake of ^{14}C by chemoautotrophic bacteria and their growth in terms of cell maintenance.

In future studies using the FDC technique, a method that may improve the incubation procedure in measuring picoplankton growth rates would be in the use of transparent semipermeable

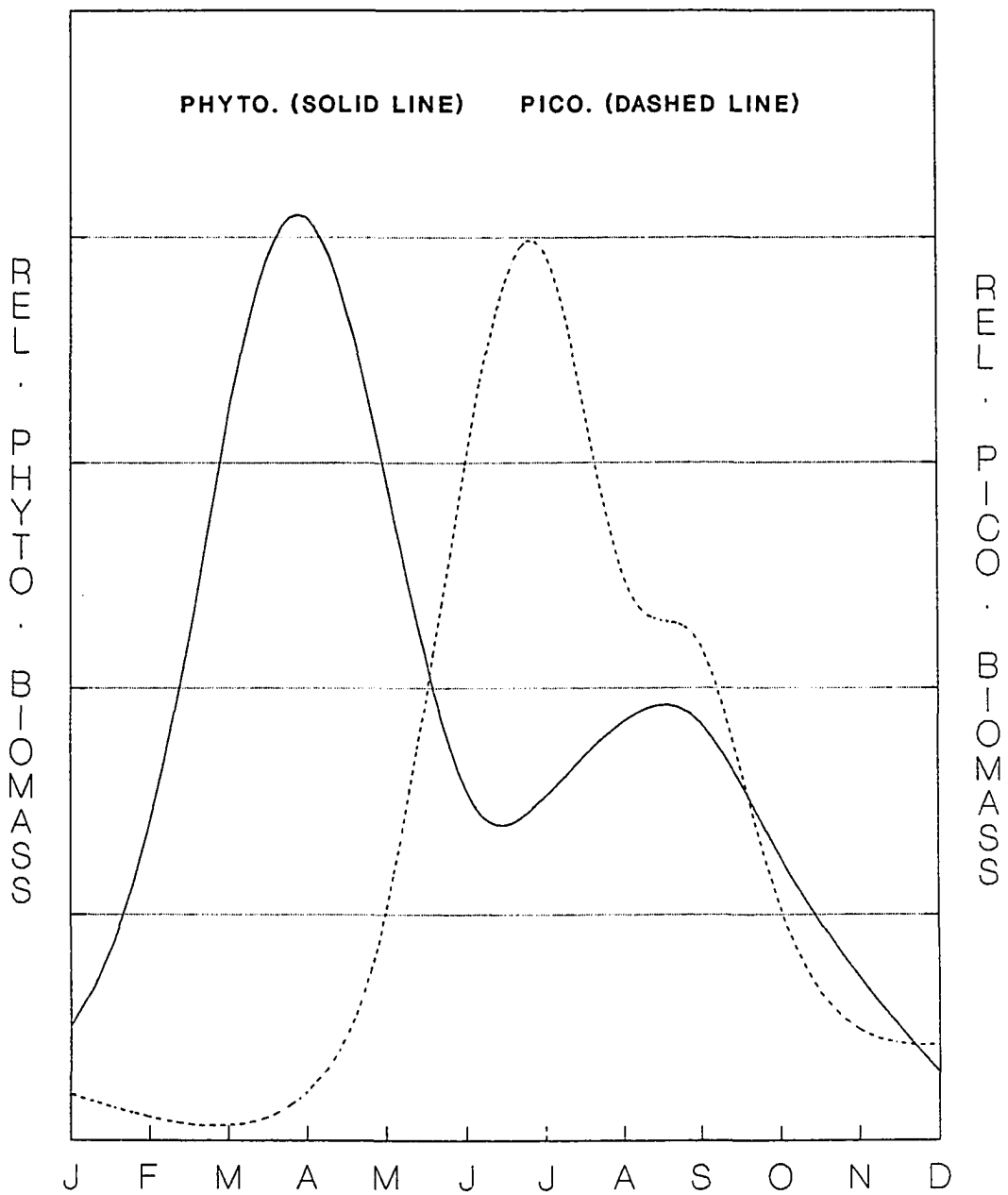
membranes as incubation chambers similar to those developed by Landry et al. (1984). Membrane pore size would need to be small enough to keep the picoplankton contained (> 0.2 μm), yet, permeable to allow the free flow of nutrients in and out of the membrane. This method would eliminate problems associated with incubating cells in closed containers and better mimic *in situ* conditions of picoplankton growth. Such modifications in FDC technique would allow for a variety of comparisons in picoplankton growth rates, where isolated components of the picoplankton (e.g., phycoerythrin dominant vs. phycocyanin dominant) can be compared to growth characteristics. Image analysis procedures would also ultimately improve the FDC technique. Total time to count picoplankton abundance would decrease, yet, there would be a need to enumerate the cells undergoing division. The specificity of the image analysis technique to distinguish cells undergoing division is low (Sieracki et al., 1985).

Seasonal patterns of picoplankton abundance reported in this study are similar to patterns of picoplankton abundance reported in the lower Chesapeake Bay and its river systems where maximum abundance is found in summer (Affronti and Marshall, 1990a, 1990b; Perkins et al., 1980). While maximum picoplankton abundance is lower than maximum abundance values reported by Affronti and Marshall (1990a, 1990b), it should be emphasized that seasonal counts reported in this study

represent picoplankton abundance from a composite of the water column. Similar patterns of picoplankton abundance have been reported in Woods Hole harbor where maximum peaks occurred in summer (Waterbury et al., 1986).

In comparing seasonal patterns of abundance between picoplankton and phytoplankton > 2.0 μm typical of the temperate north Atlantic system (Parsons et al., 1984), the peak of picoplankton biomass in summer occurs when phytoplankton biomass is low (Figure 20). Peaks of picoplankton abundance also correspond to summer peaks of zooplankton abundance in the lower Chesapeake Bay (Birdsong et al., 1988). These relationships suggest biological interactions may in part affect picoplankton abundance in the lower Chesapeake Bay and warrant further investigation. For picoplankton, competition for nutrients would decrease as zooplankton graze on the phytoplankton component. As grazing occurs, nutrients (nitrogen and phosphorous) are released through incomplete feeding making these nutrients more available for picoplankton growth (Dagg, 1974; Joint et al., 1982). The coinciding peaks of picoplankton and zooplankton abundance suggest the larger zooplankton component is not grazing on picoplankton. There is some experimental support for this conclusion based on the inability of the macrozooplankton to filter the picoplankton from the water column due to setae size (Conover, 1978; Bartram, 1980; Johnson et al. 1982).

Figure 20. Seasonal comparisons of phytoplankton (> 2.0 μm) biomass and picoplankton biomass. Phytoplankton data modified from Parsons et al. (1984).



Seasonal patterns of productivity estimated using the FDC technique revealed higher productivity values occurring in summer as physical conditions for growth (temperature, light intensity) are optimal. In contrast, lowest picoplankton productivity over the 15 month study corresponded to lower light intensities and water temperatures typical of winter. As expected, the highest amount of picoplankton activity corresponded to the highest picoplankton cell abundance during the 15 month study. Similar seasonal productivity values were reported by Joint et al. (1986) where picoplankton production in temperate waters of the European continental shelf is highest in the summer. In this study, through the use of sodium ^{14}C-fractionation techniques, over half (51.1%) of the primary productivity in the lower Chesapeake Bay was produced by picoplankton during the month of July 1989. Similar studies have reported on the significant contribution of picoplankton to primary production, yet many of these studies have occurred in oceanic environments (Gieskes et al., 1979; Johnson and Sieburth, 1979; Li et al., 1983; Smith et al., 1985; Takahashi et al., 1985). This information suggests that during the summer period when picoplankton is most active and abundant, picoplankton is a major producer of organic carbon in the lower Chesapeake Bay. This information is extremely important in modeling food web dynamics for this area of the Chesapeake Bay during this season. Picoplankton standing stock and productivity values reported in this study provide

much needed information for modeling the flow of carbon in the Chesapeake Bay ecosystem. Simple trophic level comparisons can be made more complete with this information which may ultimately contribute to resolving the fate of picoplankton carbon as a link or sink in marine ecosystems.

Understanding spatial and temporal factors influencing picoplankton within the water column is also important to consider in the overall scheme of picoplankton dynamics. Such information provides additional knowledge on the availability of picoplankton carbon over time and the physical factors involved in controlling the movement of this carbon throughout the water column. Knowing these spatial and temporal characteristics affecting the picoplankton component will lead to a better understanding of available pathways relevant for picoplankton carbon cycling in the Chesapeake Bay.

To study the spatial and temporal influences on picoplankton abundance and productivity, sampling was conducted at a high time frequency - an important consideration when working with microbial populations (Harris, 1980). Results from statistical analyses for the August diel study revealed spatial effects controlling picoplankton abundance and productivity. Average picoplankton abundance at the top sampling site was over six times greater than that observed at the bottom sampling site. The majority of picoplankton cells observed at the top sampling site were phycocyanin enriched. Average picoplankton productivity was over eight

times greater at the top compared to the bottom sampling position. Most likely, there are several different factors involved in controlling this drastic difference in cell number and cell growth from the top and bottom sampling sites. First, physical data revealed a pycnocline/thermocline region varying with depth over the 24 hour study. Based on this information, picoplankton are most likely isolated in the water mass above this density gradient. During summer, isolation of picoplankton in the upper reaches of the water column may be advantageous to some picoplankton forms as optimal picoplankton growing conditions may be more prevalent due to higher light intensities and nutrient concentrations common to the lower Bay (Birdsong et al., 1988). Morris and Glover (1981) have shown maximum rates of photosynthetic ^{14}C bicarbonate fixation in some forms of phycocyanin dominant *Synechococcus* sp. to occur at higher light intensities compared to phycoerythrin dominant forms. From these studies, phycocyanin dominant *Synechococcus* seem to be better adapted to environments where high light intensities are prevalent (i.e., surface waters). The duration of picoplankton isolation in the upper regions of the water column would be dependent on factors that disrupt pycnocline formation to include storm events, wind mixing and river flow. Picoplankton distribution in the water column has been related to the nitracline where studies have reported these areas being optimal for picoplank-

ton growth (Fogg, 1986; Olson et al., 1990).

In addition to spatial influences, picoplankton productivity and abundance is also affected by a temporal component. Picoplankton abundance at the bottom sampling site during the August diel study was affected over time with Tukey's aposteriori tests revealing a significant increase in picoplankton abundance at times which corresponded to the ebbing tide. During ebb tide, higher concentrations of picoplankton in less saline water passed over the bottom sampling site due to entrainment and a major increase in picoplankton abundance resulted. Only the bottom sampling site revealed statistically significant changes in picoplankton abundance over time as picoplankton abundance at the top sampling site was more homogeneous. There was less variation in salinity at the top sampling location over time compared to the bottom sampling site. A more productive picoplankton component was observed associated with the lower saline environments as picoplankton productivity also increased with the ebbing tide. Higher productivity values may be explained by higher nutrient concentrations and higher light intensities characteristic of the lower saline water as its less dense waters flow over more dense saline waters in estuarine environments at this time of the year. Certain varieties of phycocyanin enriched *Synechococcus* sp., more typical of fresh water environments, are more productive and better adapted to high light intensities

compared to phycoerythrin enriched picoplankton cells more typical of saline environments (Morris and Glover, 1981; Alberte et al., 1984; Wood et al., 1985) .

During the winter diel study, an opposite pattern of picoplankton abundance and productivity, in relation to spatial and temporal factors, was evident in comparison to summer. A more homogeneous mixture of picoplankton cells in the water column was observed compared to summer with the bottom sampling site now showing higher average picoplankton counts over the 24 hour study. A pycnocline was not present during this 24 hour study. When the pycnocline is not present, there is a greater chance for picoplankton to be distributed throughout the water column. Takahashi and Bienfang (1983) reported picoplankton sinking rates to be extremely slow in a study of phytoplankton biomass and photosynthesis in subtropical Hawaiian waters. Fogg (1986) calculated the sinking rates of picoplankton using Stoke's Law to be 2.5 mm per day. With this information, picoplankton are not expected to settle from the top of the water column to the bottom sampling site when there is no pycnocline; rather, water mass movements would be more effective in the distribution of picoplankton carbon (Raven, 1986). There have been reports of picoplankton sinking out of the water column via zooplankton fecal pellets and macroaggregates (Glover, 1985). This sinking phenomenon would contribute to the availability of picoplankton carbon to the benthos (Stockner and Antia,

1986). Picoplankton productivity was shown to be affected by sampling position where the bottom sampling site showed higher productivity compared to the top.

Picoplankton abundance and productivity were affected by a temporal component in the winter sampling period. Both top and bottom sampling sites revealed an increase in picoplankton abundance with the flooding tide. As the more saline water passed over the sampling positions, there was an increase in picoplankton abundance. The higher picoplankton abundance in more saline waters during winter partly explains higher average picoplankton counts at the bottom sampling site compared to the top, as average salinity values increased with depth. A more productive picoplankton component was observed in the higher saline waters during winter. Picoplankton productivity at the upper sampling site decreased drastically during the ebbing tide where picoplankton in less saline waters contributed to lower productivity values. These data suggest a more stable picoplankton population in more saline water where the phycoerythrin enriched cells appear to be more tolerant of colder temperatures compared to phycocyanin picoplankton typical of fresh water environments.

Proposed trophic interactions

The phototrophic picoplankton are producing a significant amount of carbon seasonally in the lower Chesapeake Bay. Yet, questions still remain as to the specific processes involved

in controlling the fate of phototrophic picoplankton carbon in this dynamic ecosystem. From the results of this study, a logical argument is made that the utilization of picoplankton carbon varies and is broadly influenced by the time of year and more specifically by local physical parameters influencing the distribution and availability of picoplankton in the water column. High frequency studies similar to this one offer a direct approach in influencing the patterns picoplankton experience seasonally.

If it is assumed picoplankton patterns observed for the high frequency studies of August 1988 and January 1989 represent typical patterns of picoplankton abundance and productivity for these times, there would be then an alternating pattern of picoplankton carbon abundance throughout the year. During this alternating pattern, the majority of existing picoplankton carbon would be located at different locations within the water column, at different seasons of the year. As the picoplankton carbon availability changes within the water column, so might the many factors and processes determining picoplankton carbon flow in estuarine environments.

Since the majority of picoplankton standing stock over the year is located above the pycnocline during the summer period, the fate of picoplankton carbon will be markedly controlled by two processes identified to occur in this portion of the water column. First, grazing of picoplankton

by protozooplankton will include heterotrophic microflagellates (Sieburth and Davis, 1982; Davis and Sieburth, 1984) and mucous net feeders, such as salps (Pace et al., 1984). This pathway has been proposed as a link to higher trophic levels. Second, organic material produced from picoplankton is a source for heterotrophic bacterial degradation in the water column. To what extent each of these pathways for picoplankton carbon is involved in the flow of carbon in this estuarine system requires further experimental study.

In winter, the majority of picoplankton carbon is more available to bottom portions of the water column. During this period, picoplankton may be more important in providing nutrients to benthic populations, including species that are deposit feeders and suspension feeders. A seasonal shift in importance from picoplankton carbon flow in the upper regions of the water column to the lower portions of the water column would be expected. With this shift in carbon flow, it is hypothesized that the efficiency at which picoplankton carbon is transferred to higher trophic levels will also change as different organisms and pathways become involved during this season. Organic material produced from picoplankton would also be a source for heterotrophic bacterial degradation in the sediments. However, this action would influence the nutrient dynamics and physical factors effecting the benthos (ie., hypoxic and anoxic conditions) ultimately affecting water quality. During those transitional seasons (spring and

fall), picoplankton carbon is likely available for both top and bottom processes due to the mixing of the water column. Sediment trap experiments would be useful in calculating the amount of phototrophic picoplankton carbon produced from the upper regions of the water column that would reach the lower portions of the water column during less stratified conditions. This information would provide more insight regarding the fate of picoplankton carbon in the estuarine environment and its availability to different areas of the water column.

To more fully understand the role picoplankton have in the estuarine environment, holistic studies similar to Davis et al. (1985) and Landry et al. (1984) involving all elements of the marine environment in conjunction with picoplankton dynamics would be essential. Ideally, a mesocosm study conducted to investigate all components that would influence picoplankton dynamics on time frequencies similar to this study would be useful. However, the logistics of time and cost of such a study may be limiting factors. Yet, since a technique like frequency of dividing cells is more cost effective, its application to these studies is very feasible.

Chapter 6

CONCLUSIONS

Phototrophic picoplankton dynamics in the lower Chesapeake Bay were studied for 15 months from June 1988 to October 1989 using epifluorescence microscopy, frequency of dividing cells and sodium ^{14}C-bicarbonate techniques. The regression equation: $\mu = 2.37 \times 10^{-3}$ (FDC) + 0.024 explained the relationship between frequency of dividing cells and the phototrophic picoplankton growth rate. By using the FDC method developed from *in situ* incubations of natural picoplankton populations, a quick estimate of picoplankton productivity can be obtained based on direct counting procedures. Taking into account factors affecting duration of cell division, the frequency of dividing cells technique is shown to correlate highly with sodium ^{14}C-bicarbonate fractionation techniques and may be used as an alternative method in measuring phototrophic picoplankton productivity.

Phototrophic picoplankton abundance varied over the 15 month study with maximum abundance occurring during both summers of the study. Phototrophic picoplankton productivity varied with maximum productivity also occurring in the summer months. From this study, phototrophic picoplankton in the

Chesapeake Bay were the major summer producers of organic carbon when over half (51.1%) of the total primary productivity was due to this phototrophic component.

The amount of picoplankton standing stock and productivity varied seasonally at different depths of the water column. In addition, the picoplankton carbon was controlled both spatially and temporally over the year by local physical factors. In summer, tidal flow and pycnocline formation affected the location of picoplankton carbon. In winter, only tidal flow influenced picoplankton carbon flow in the lower Bay. Tidal flow and pycnocline formation influence the availability of picoplankton carbon to various segments of the water column where a variety of pathways for picoplankton carbon exist and most likely change in their importance over the year.

In comparing summer and winter diel studies, higher phototrophic picoplankton abundance and productivity were found in summer where higher phototrophic picoplankton abundance and productivity occurred at the top sampling depth. During the summer, phycocyanin enriched *Synechococcus* dominated the picoplankton composition. In winter, higher phototrophic picoplankton abundance and productivity were associated with the bottom sampling depth, where phycoerythrin enriched *Synechococcus* were the dominant picoplankton.

McCarthy et al. (1974) reported on the importance of nanoplankton (cells < 20 μm) in the Chesapeake Bay and how this one component was responsible for a high percentage of primary productivity. The results of this study recognize the importance of a smaller component, the picoplankton, as a producer of organic carbon in the lower Chesapeake Bay during the summer. Rather than larger phytoplankton cells as being the only focus in annual Bay trophodynamics, the microbial component is seasonally a major autotrophic component in the Bay. As the Chesapeake Bay becomes more eutrophic, trophic shifts may become more prevalent and this microbial component may become more instrumental in regulating energy and nutrient flow in this ecosystem (Greve and Parsons, 1977).

Results of this study have provided base line information on the phototrophic picoplankton dynamics in the lower Chesapeake Bay. These and future data on picoplankton standing stock and productivity will provide information for a more complete analysis of ecosystem models for studying carbon flow in the lower Chesapeake Bay. With further understanding of the factors controlling the availability of picoplankton in the water column, additional studies can more precisely determine the fate of picoplankton in this estuary.

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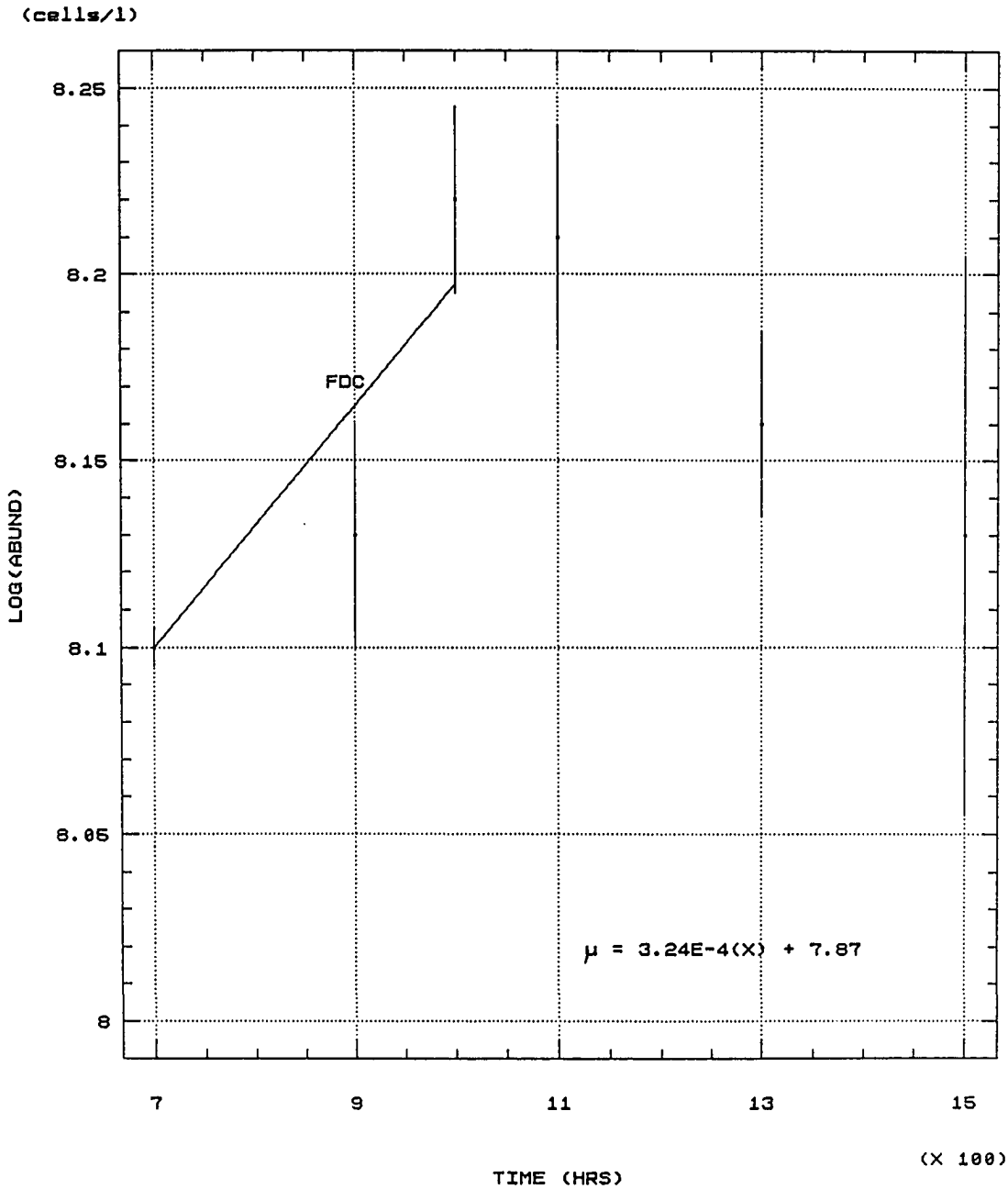
Appendix A. *In situ* incubations for June 1988 through October 1989 (August 1988 and January 1989 not taken). Growth during the exponential growth phase is expressed by best fit line (equation shown). FDC indicates time at which maximum frequency of dividing cells was observed.

PLOT OF TIME VS. ABUND. FOR JUNE



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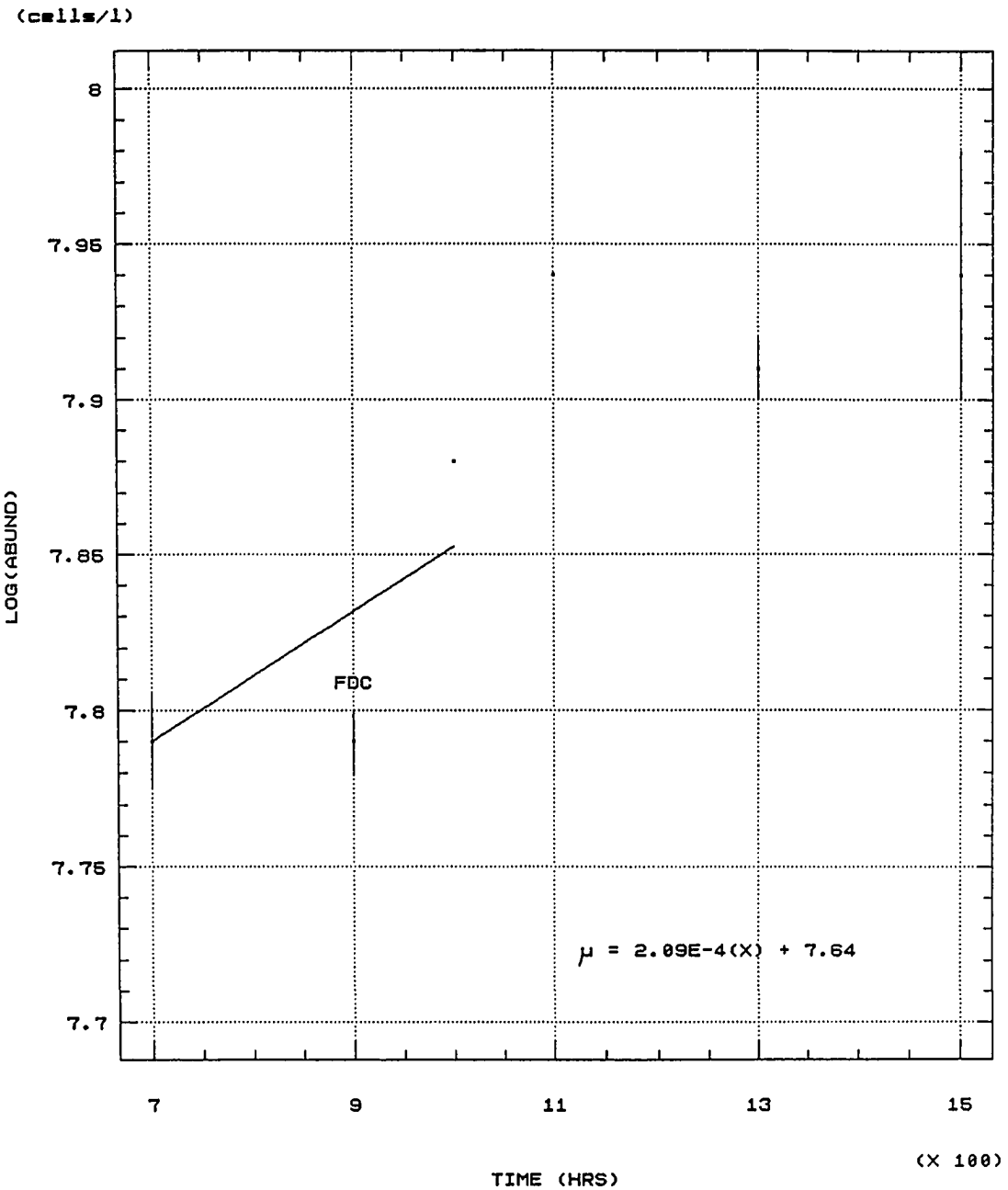

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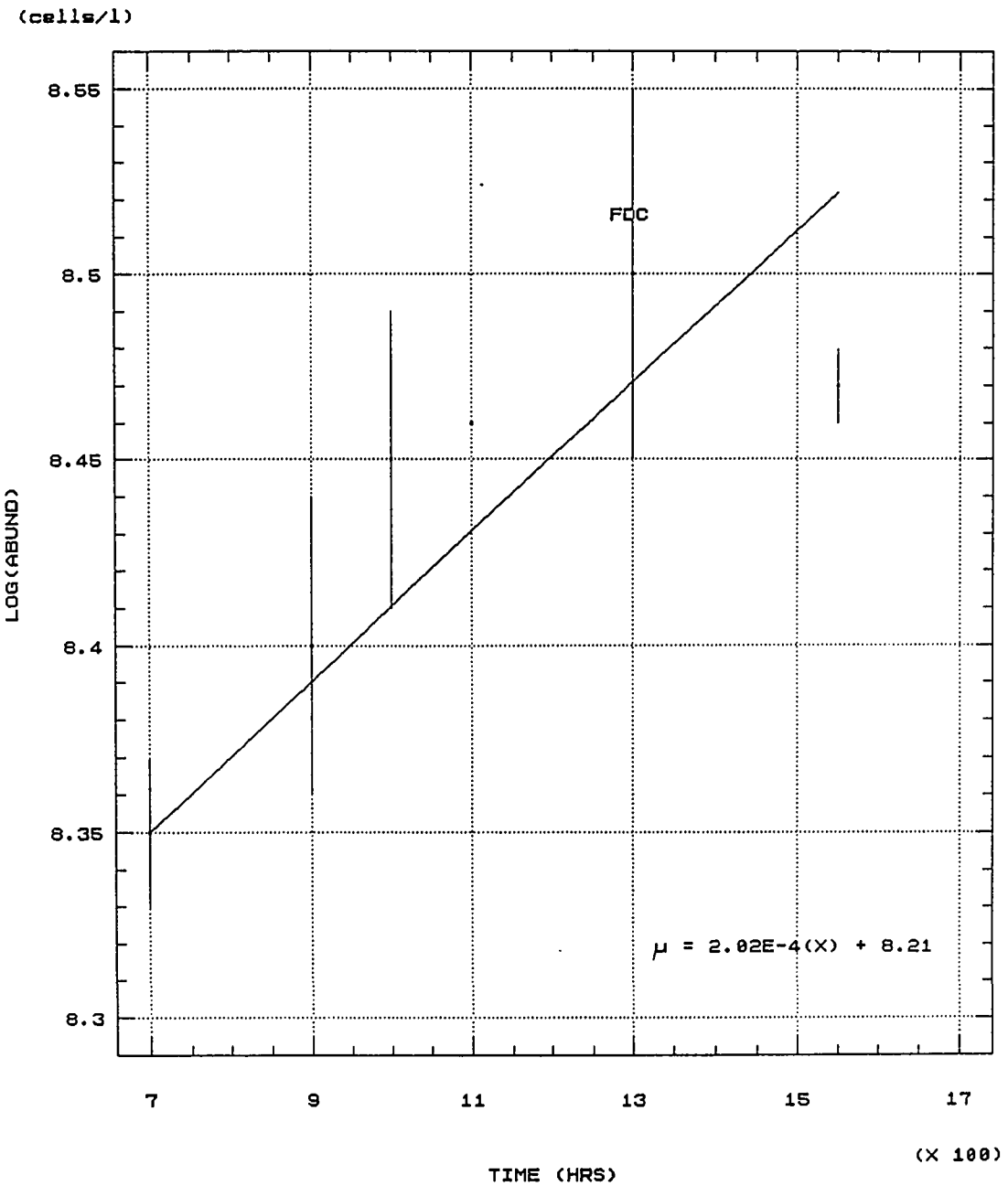

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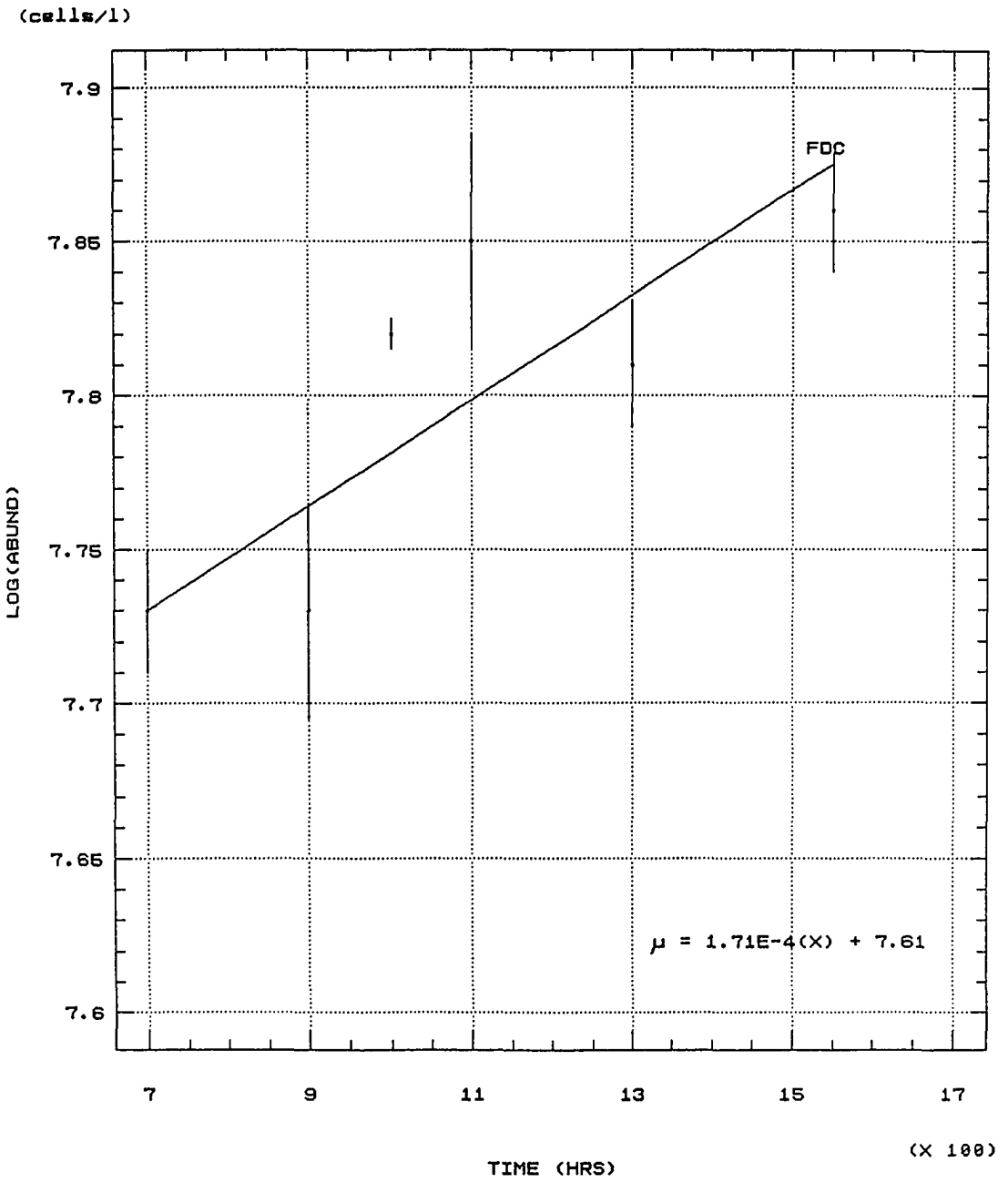

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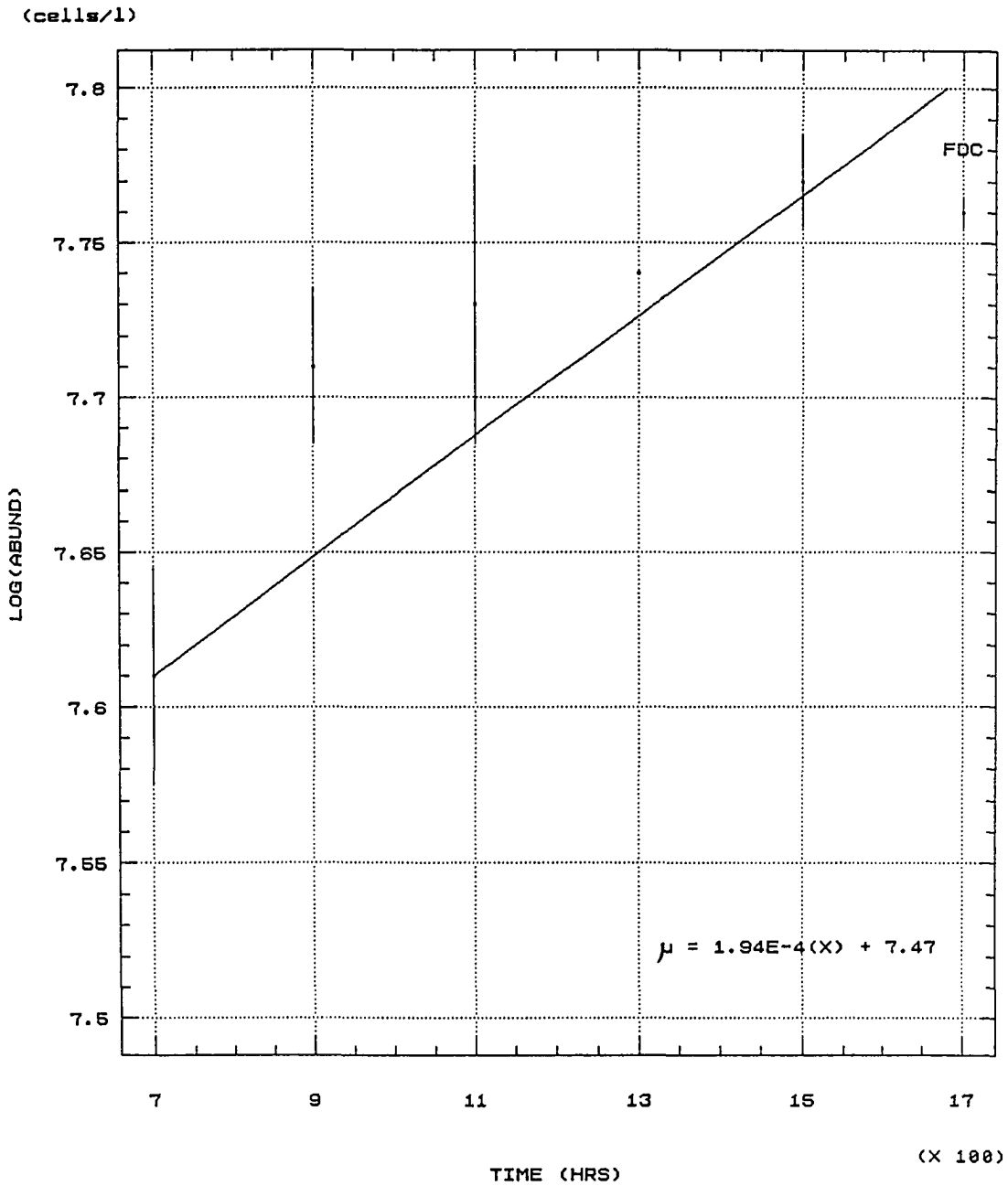

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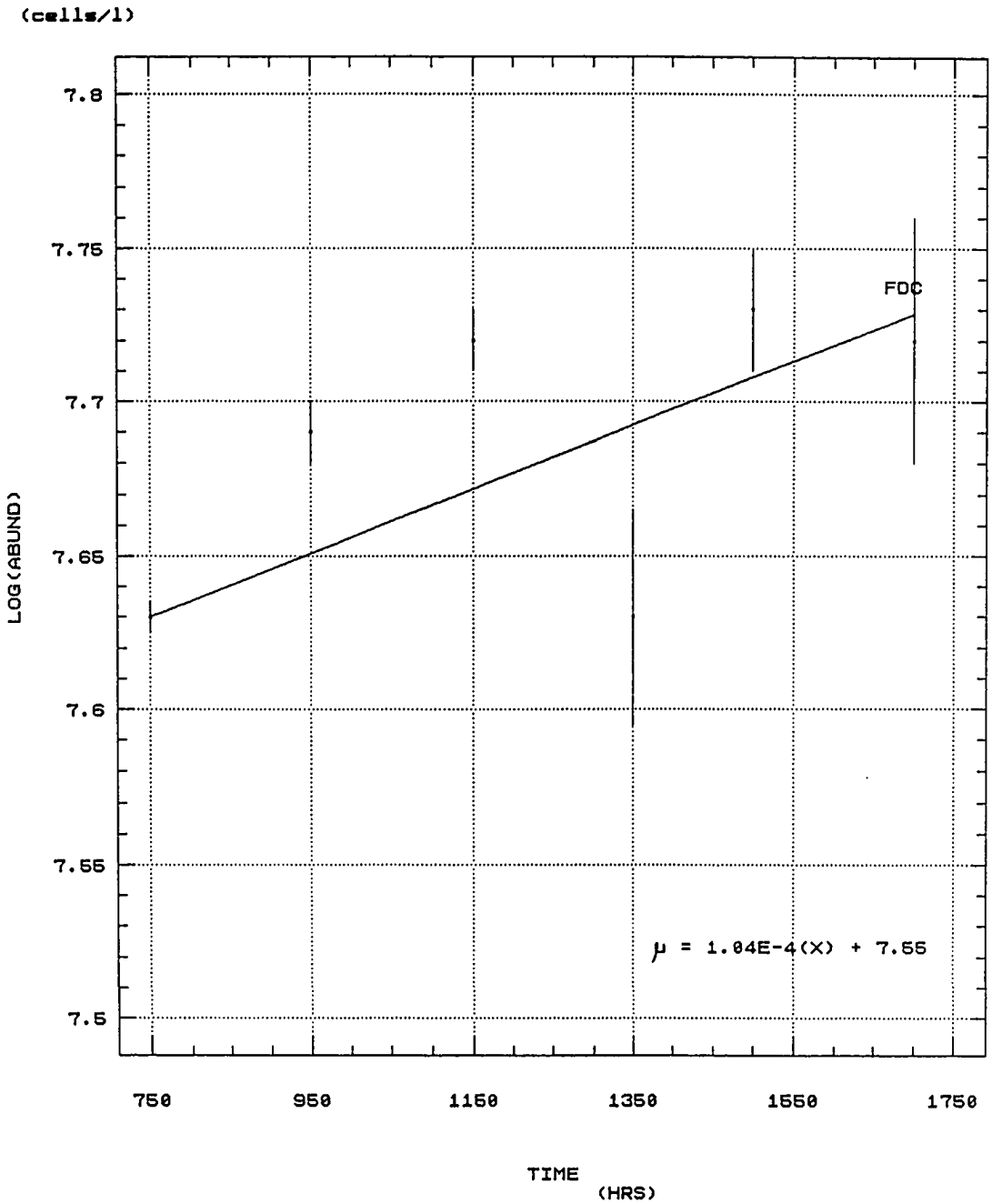

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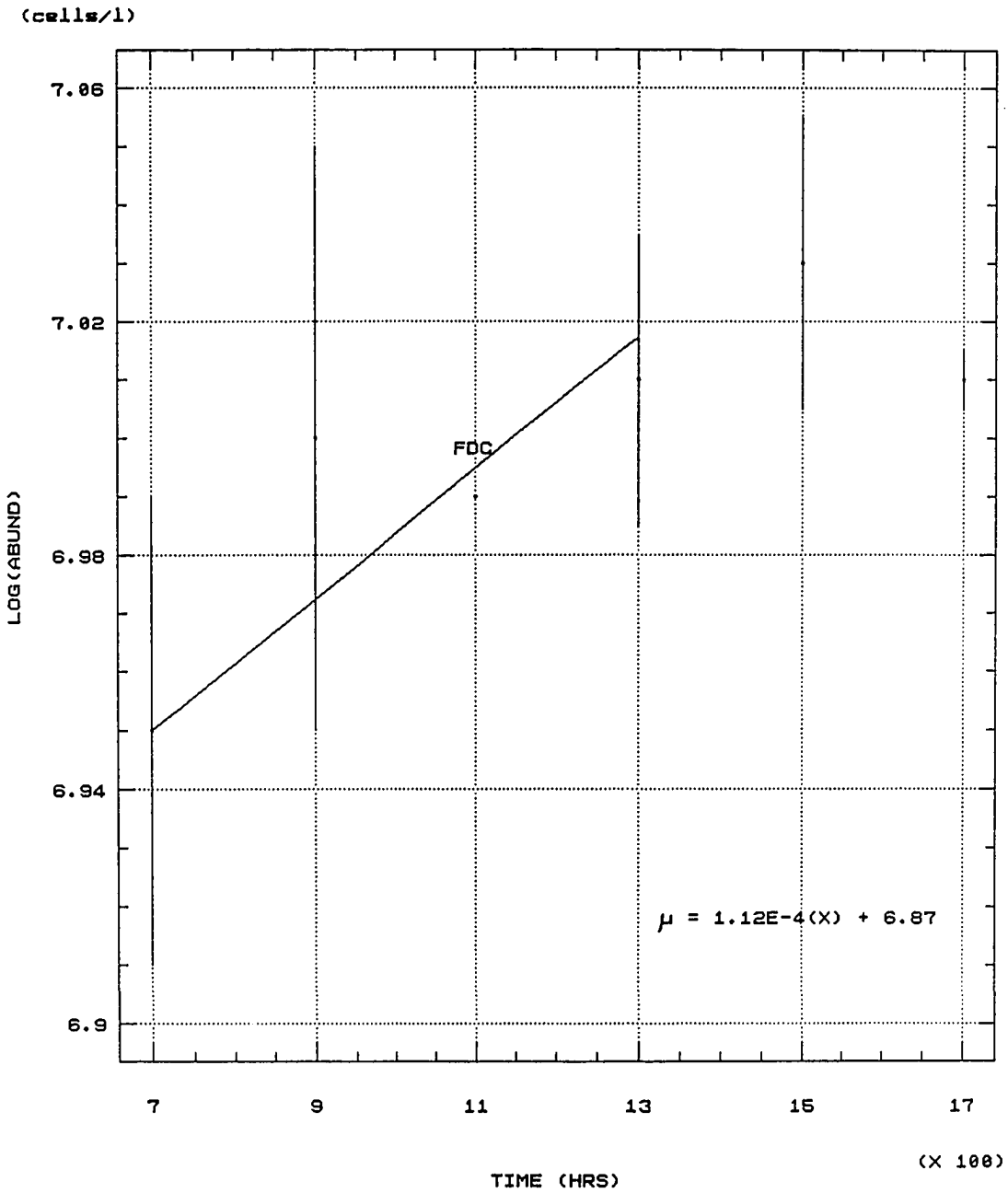

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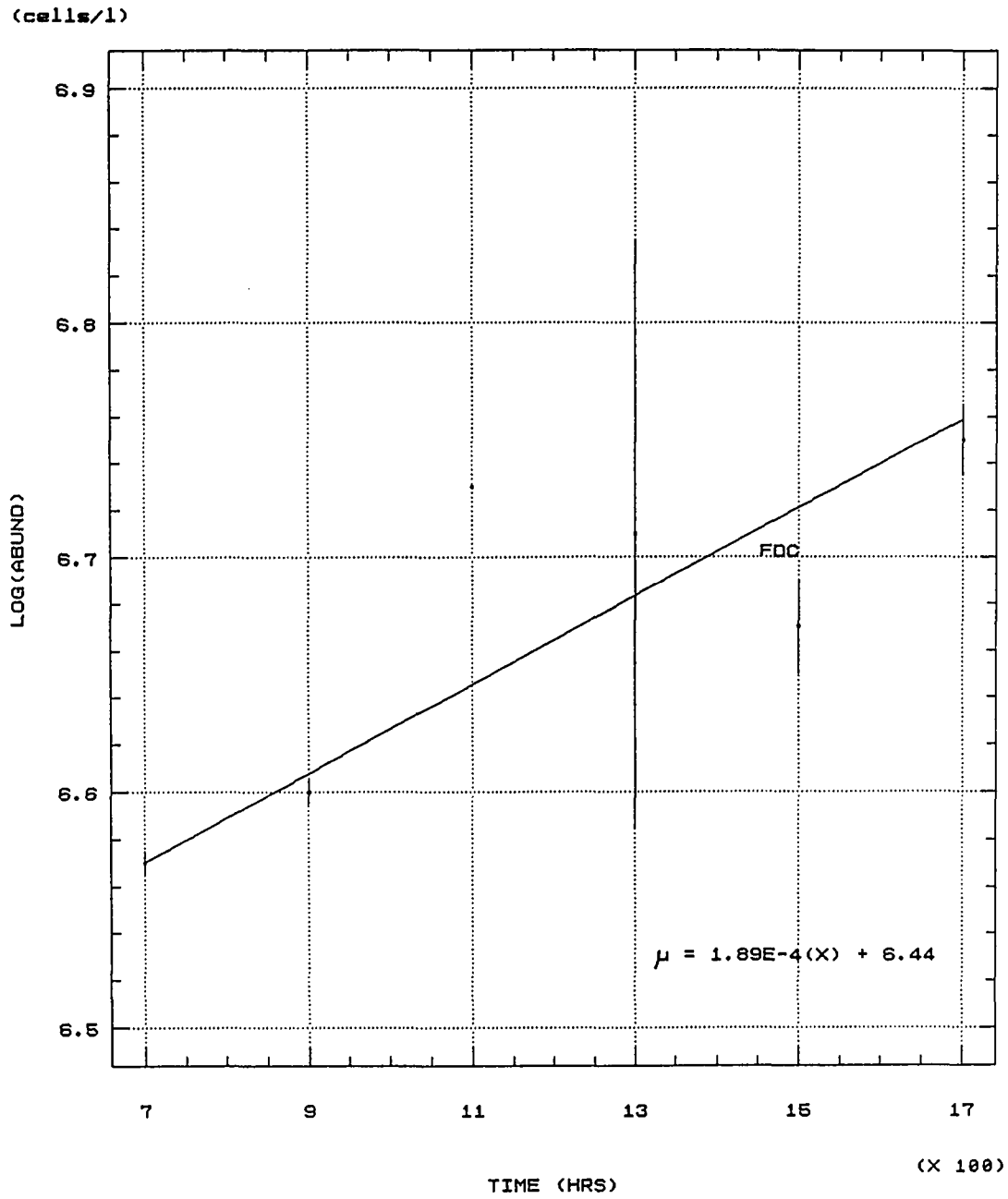

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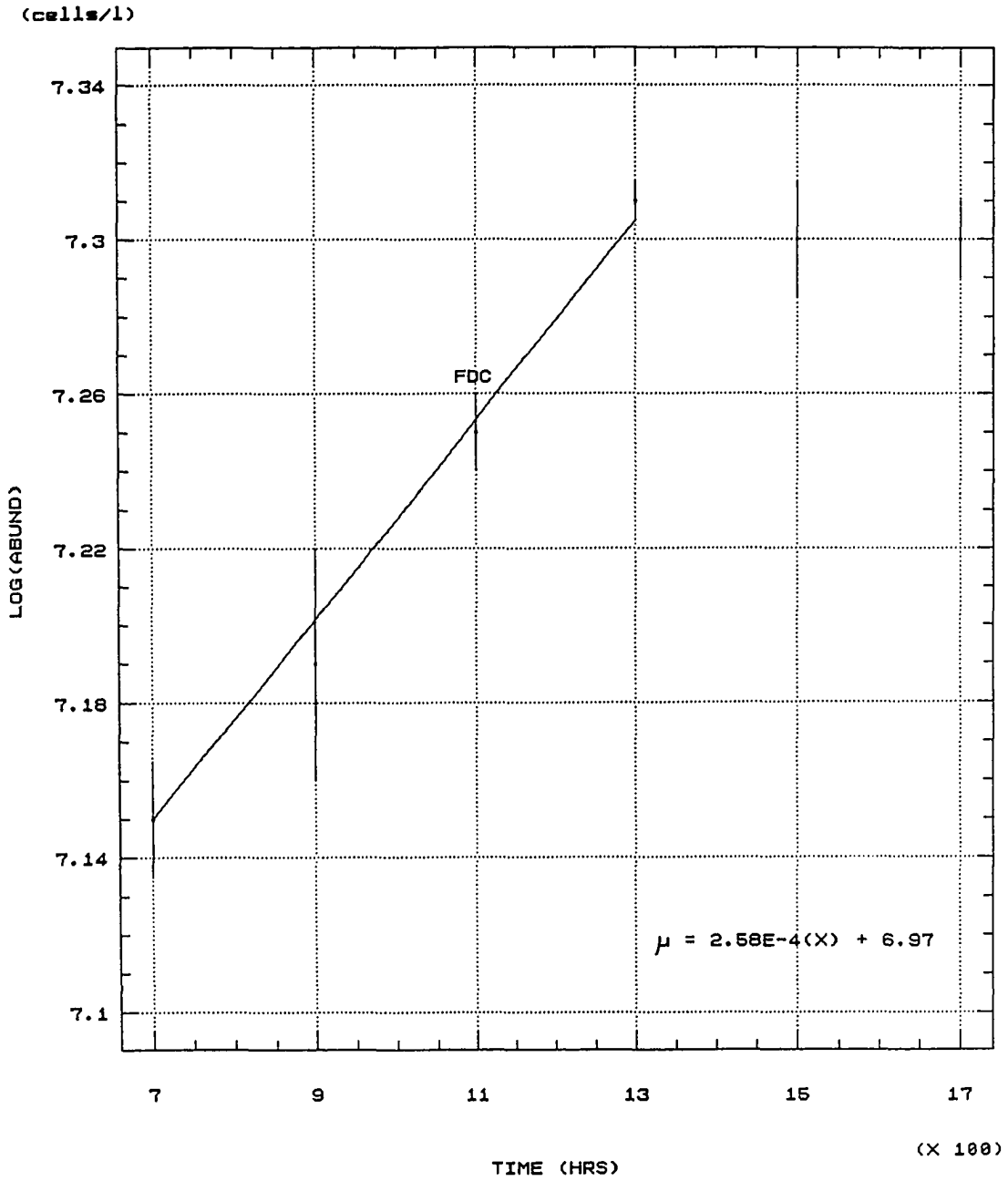

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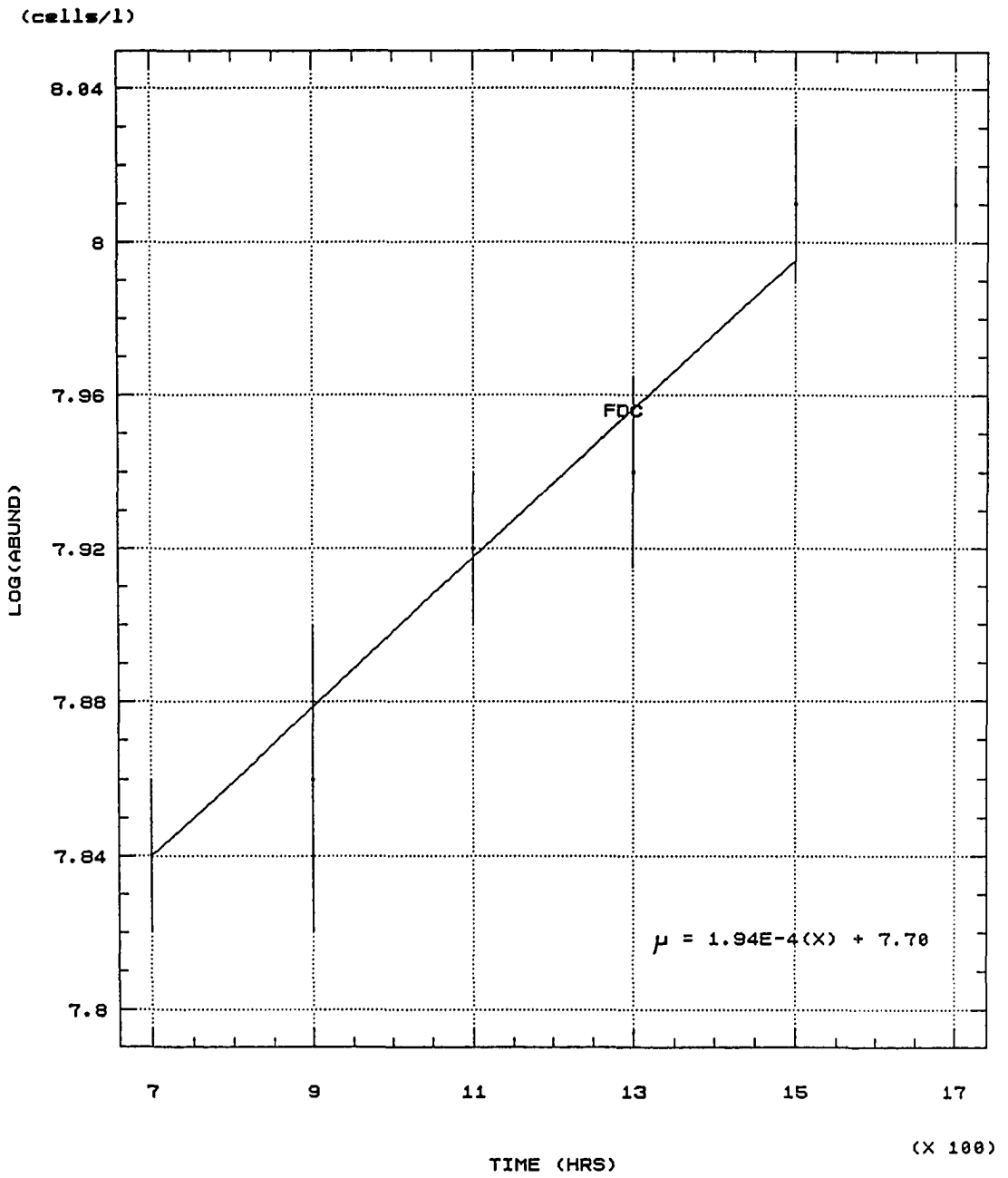

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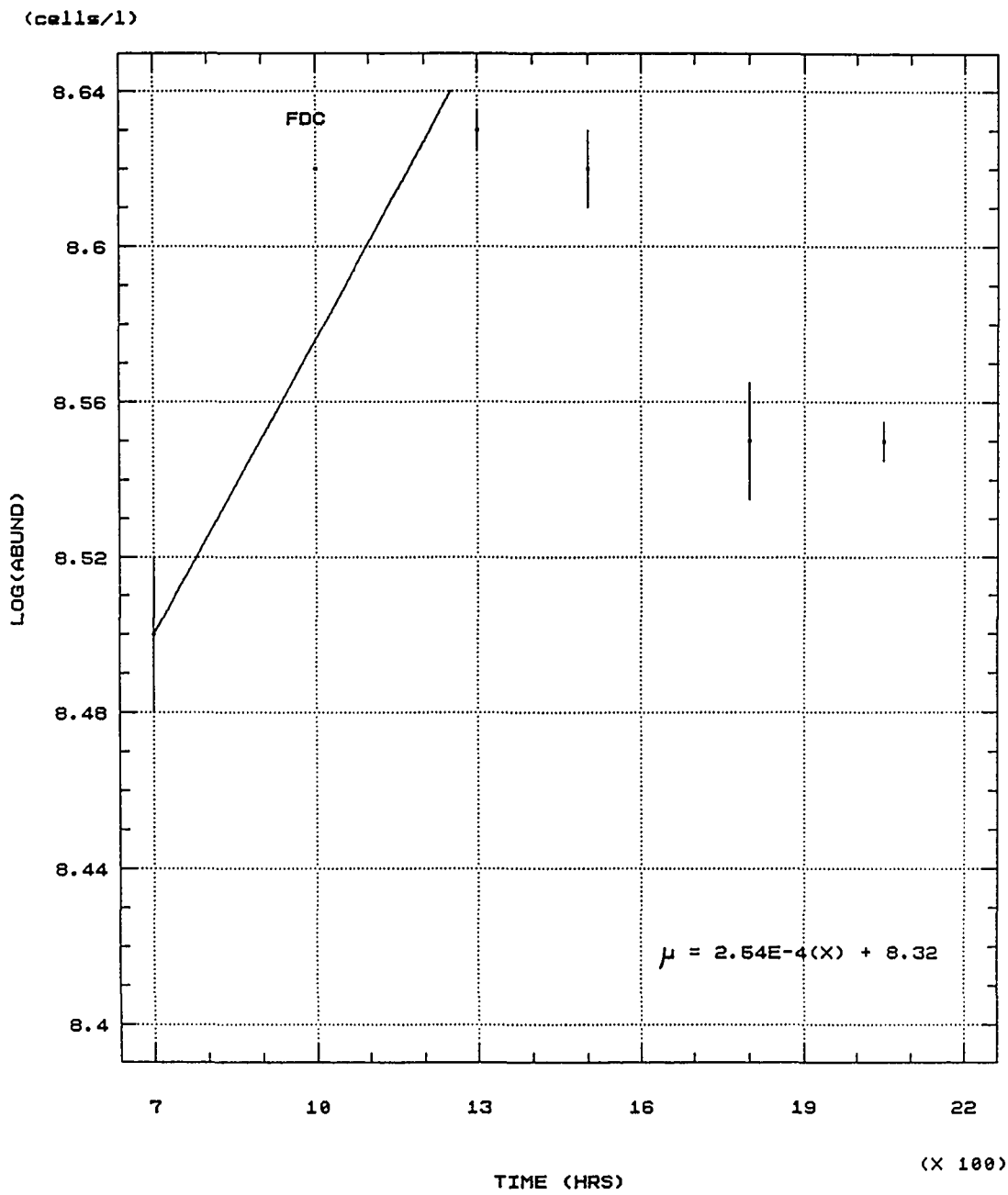

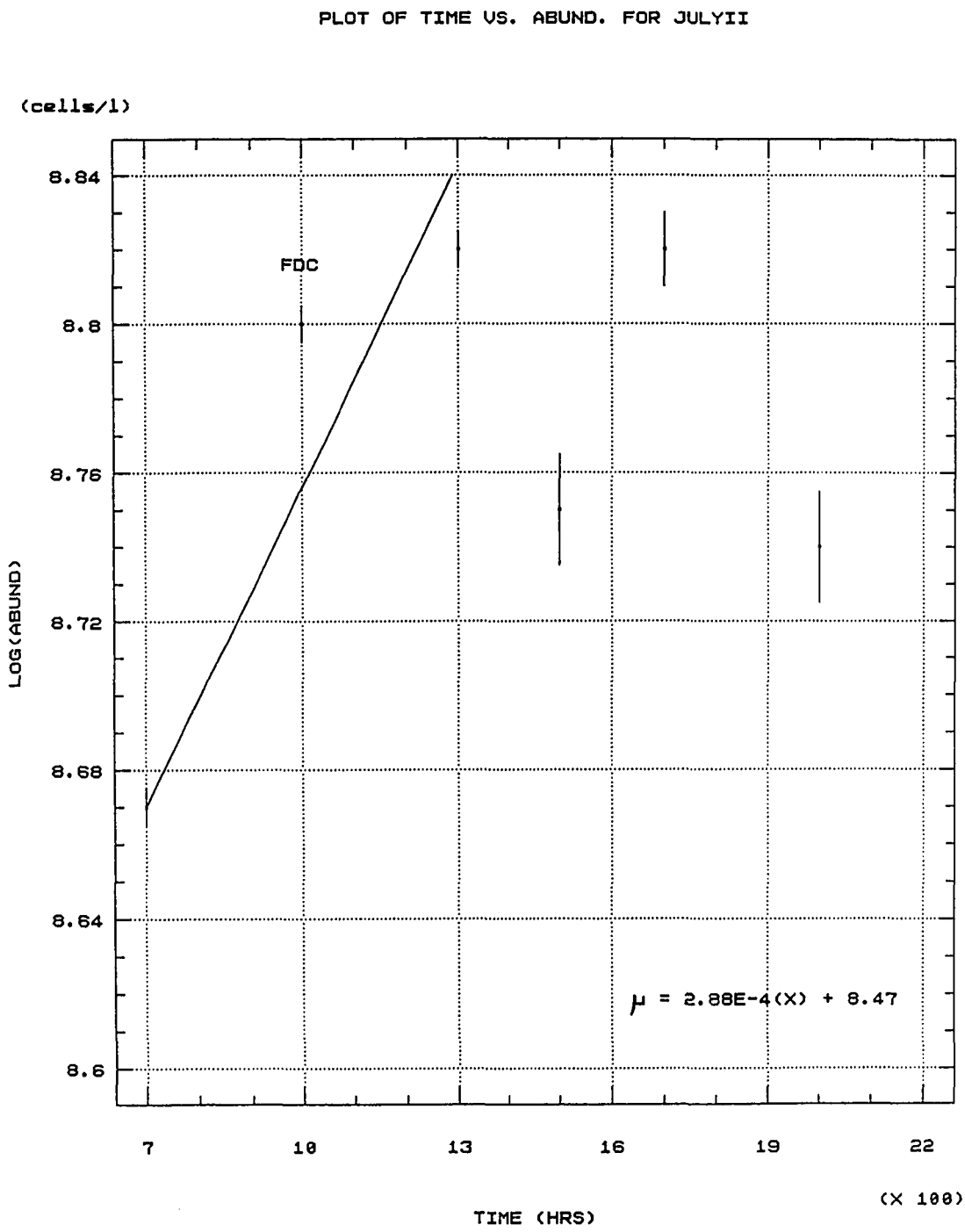

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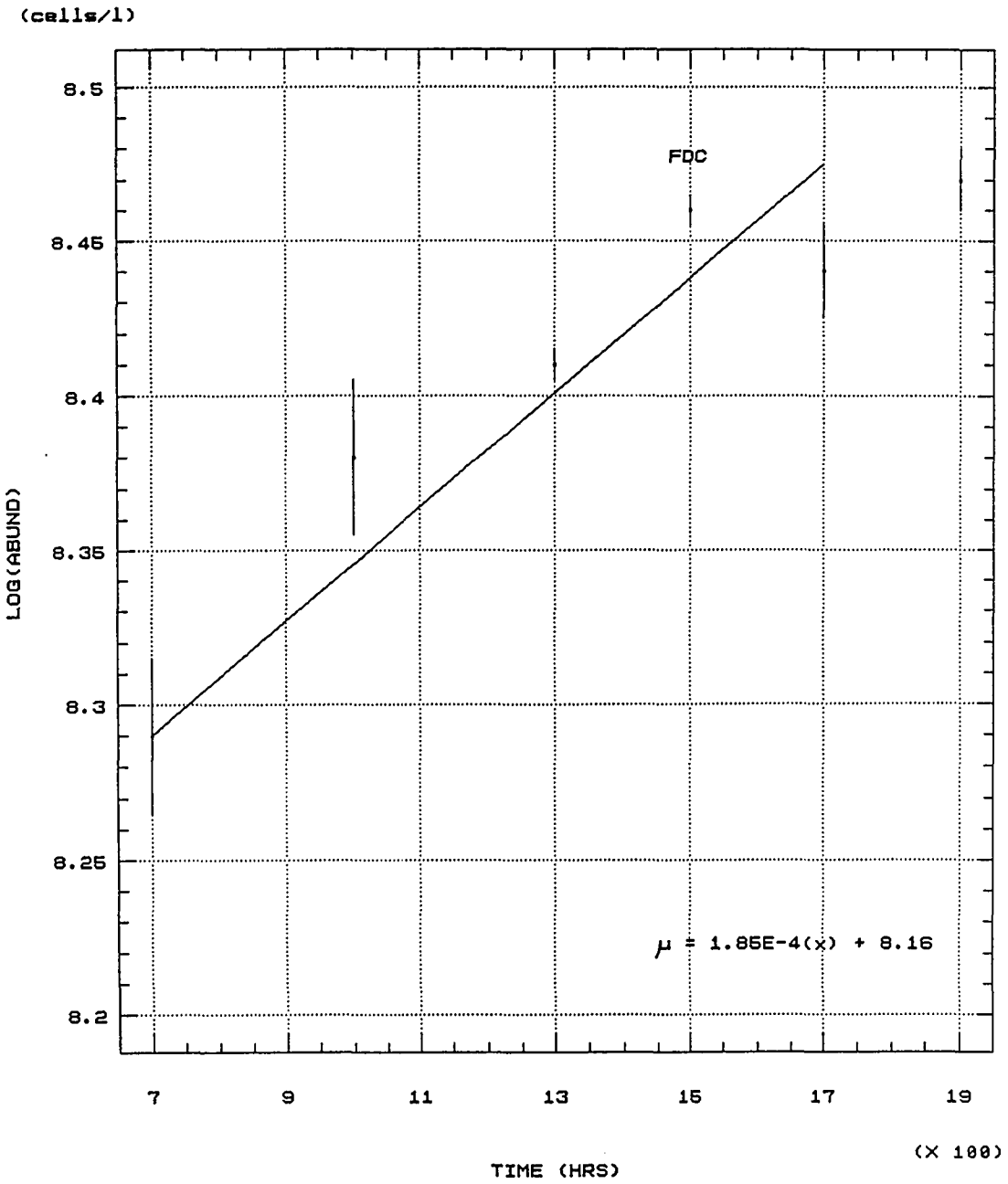

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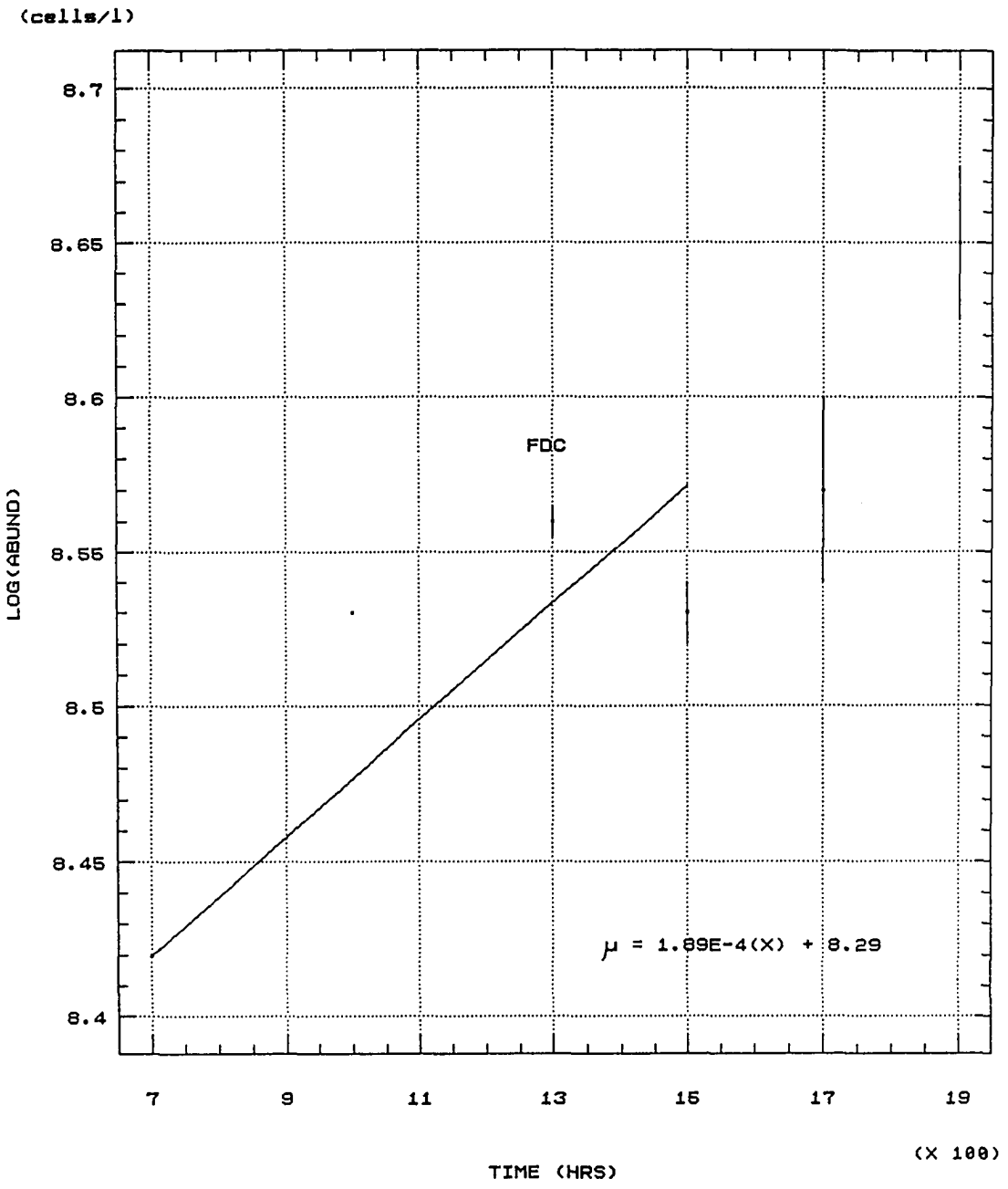
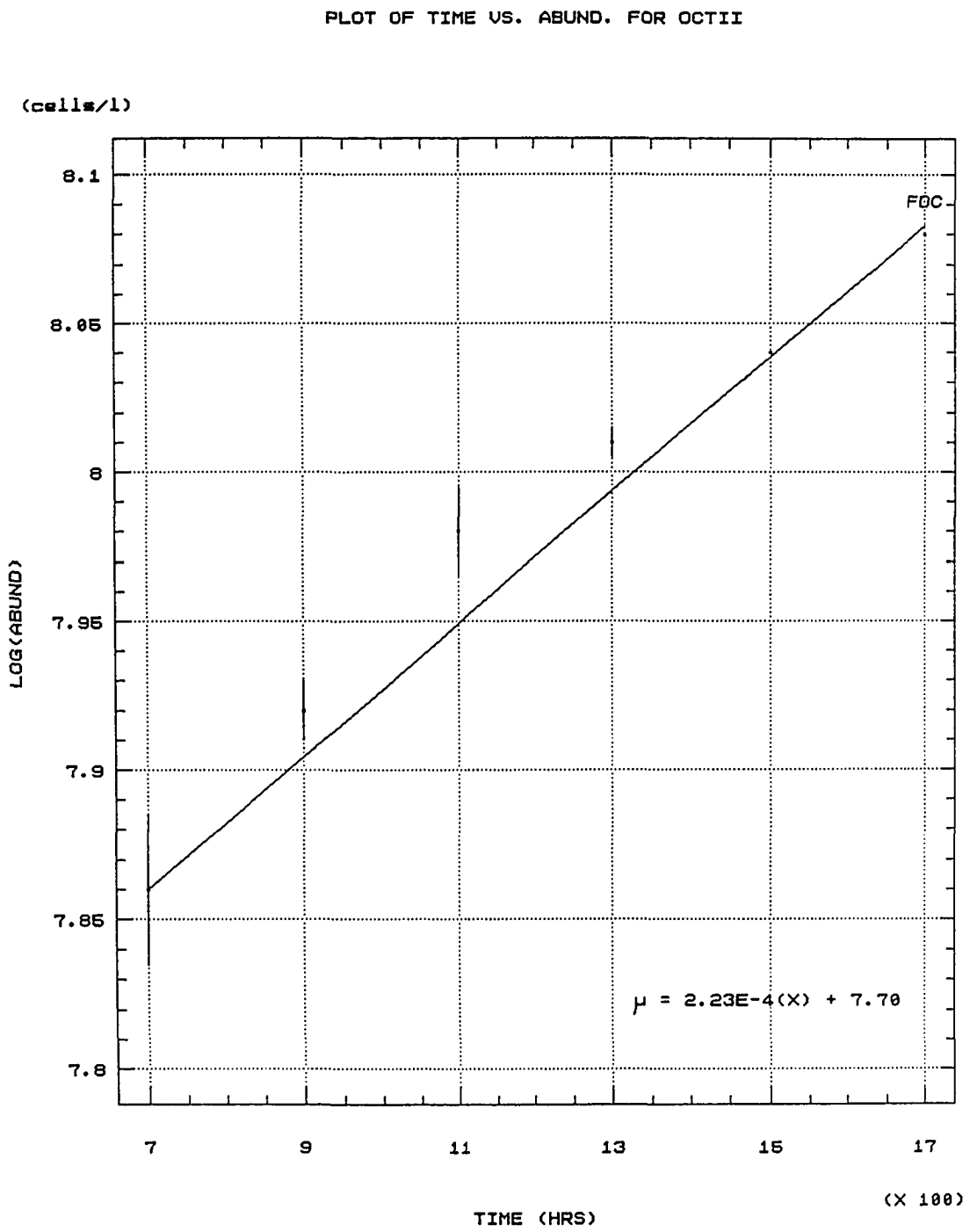
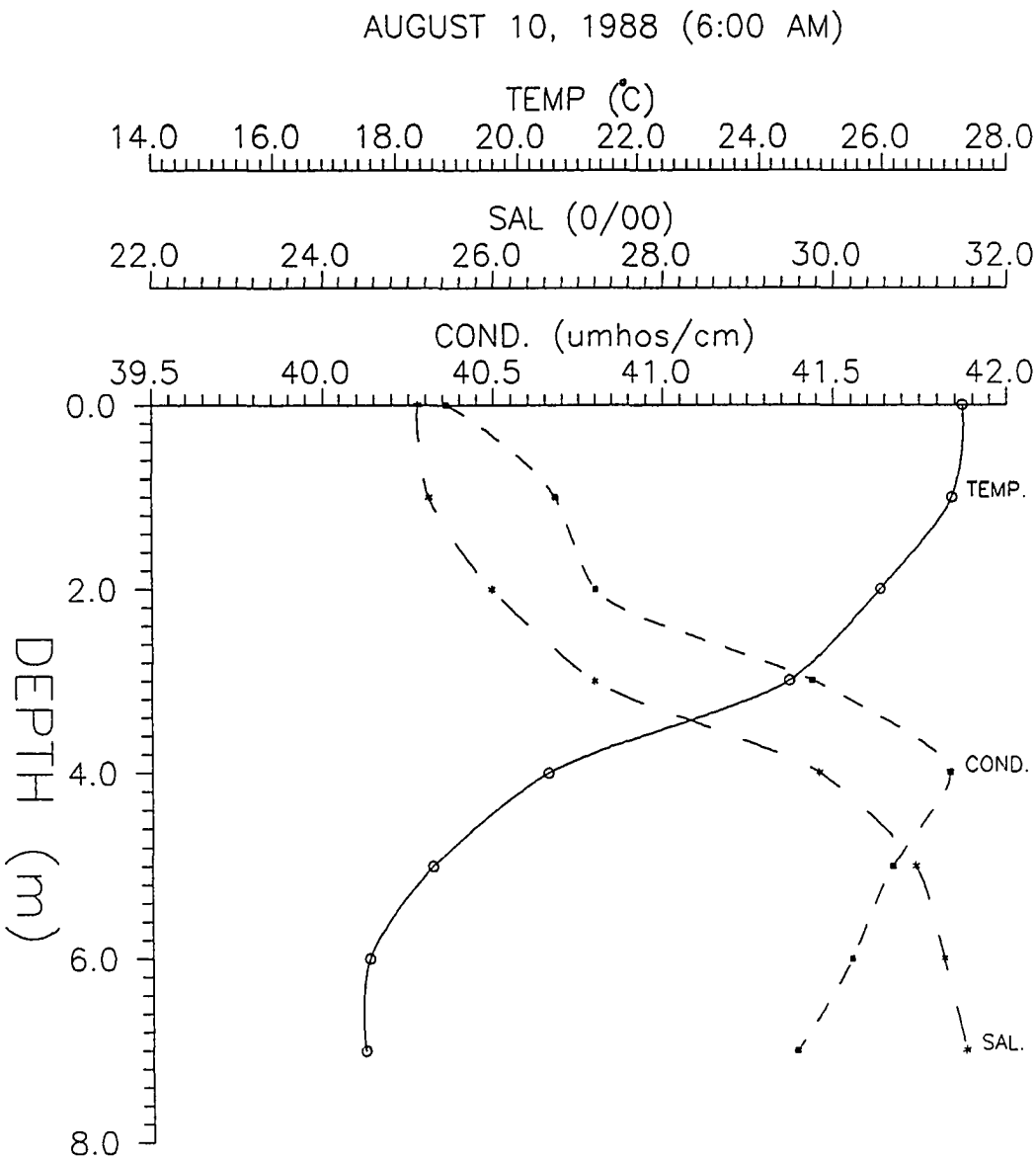
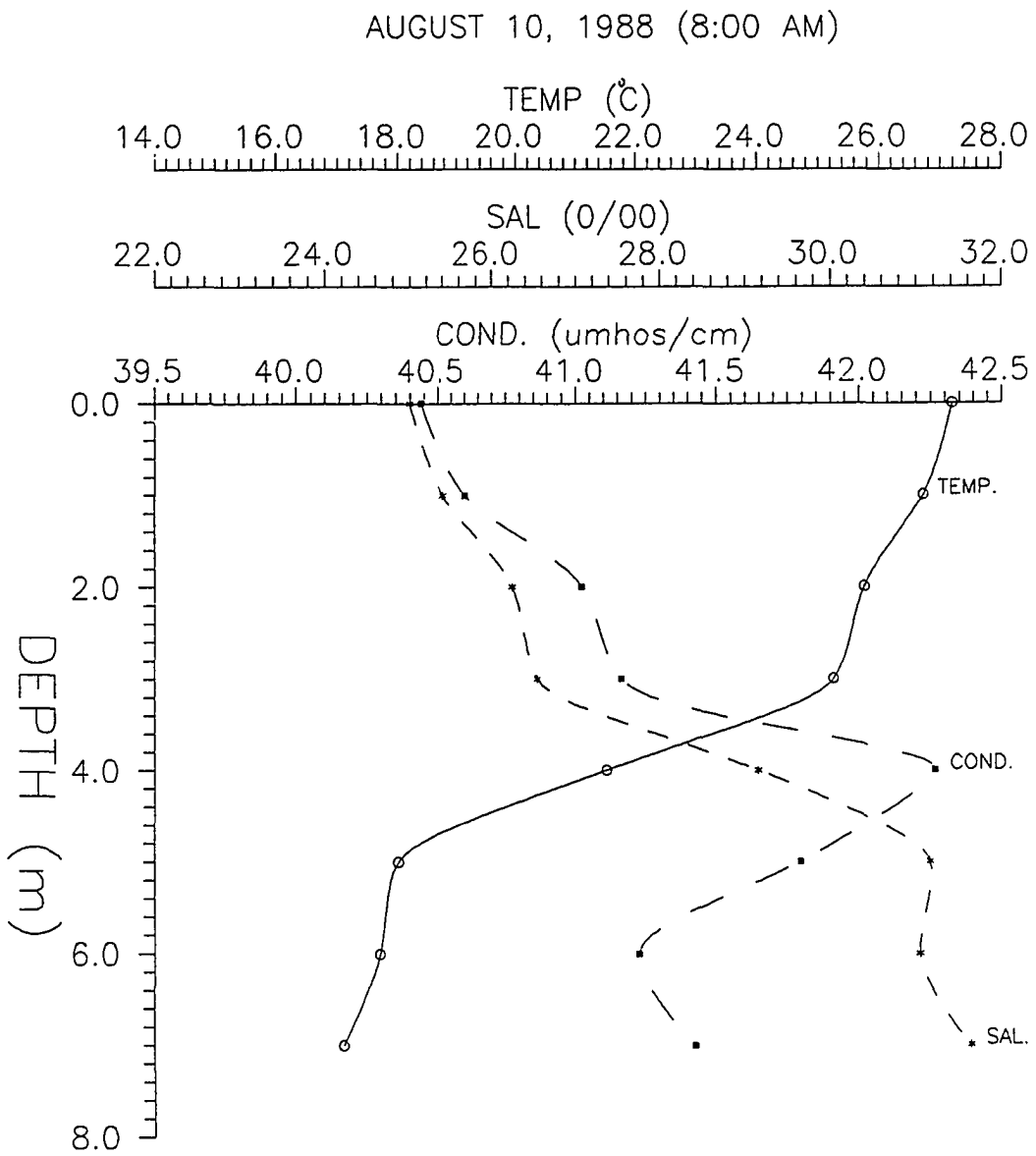
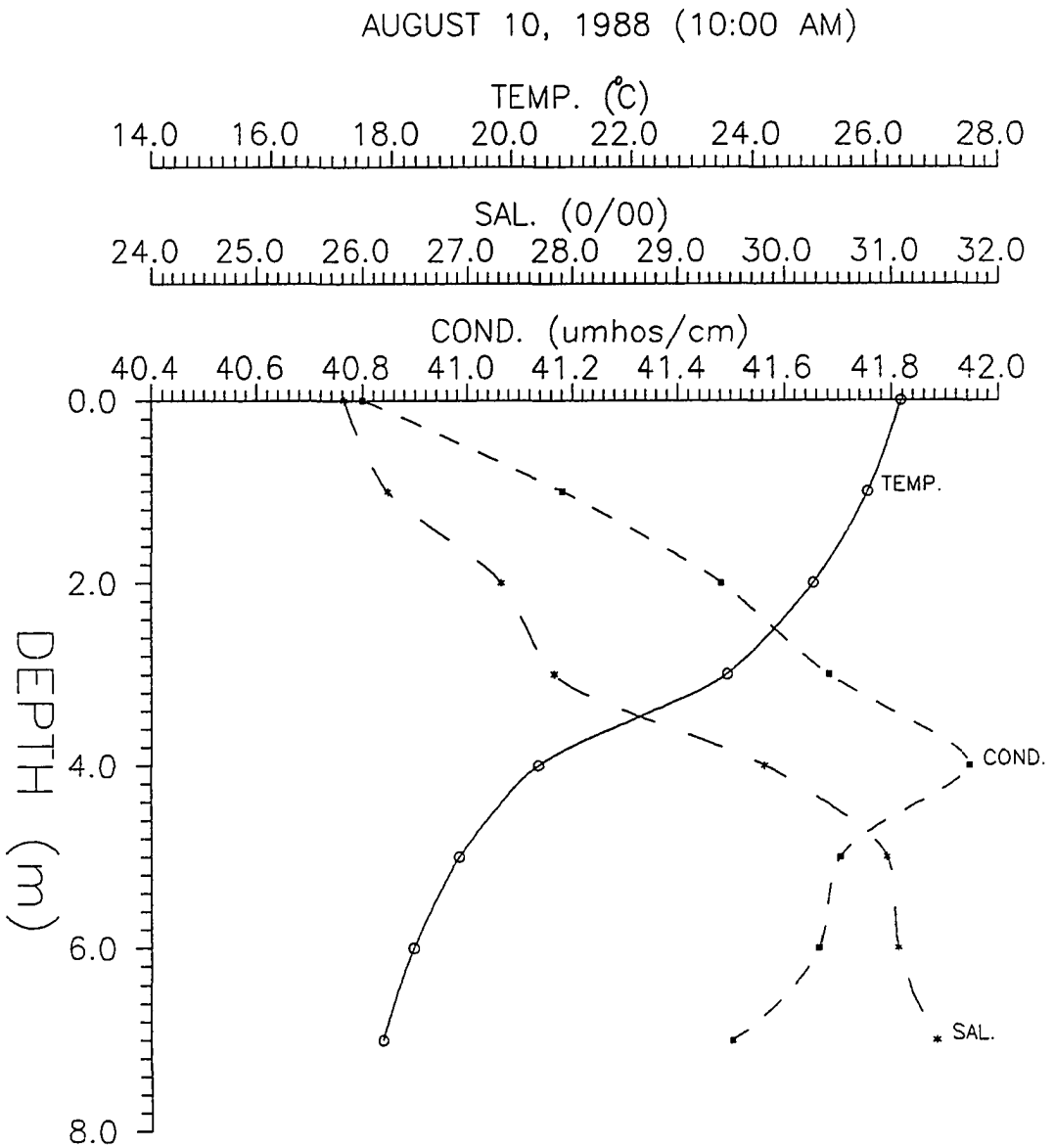


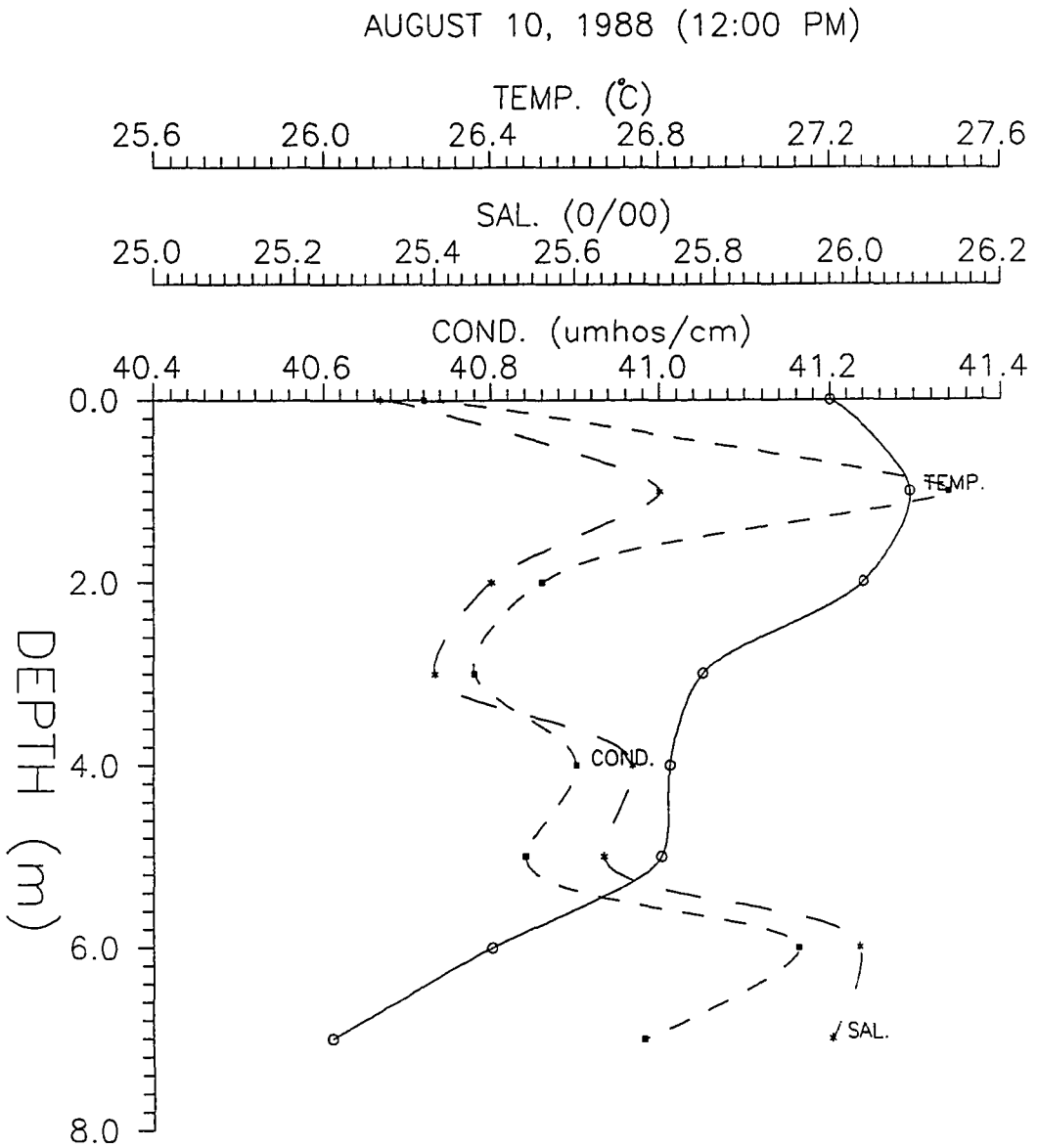


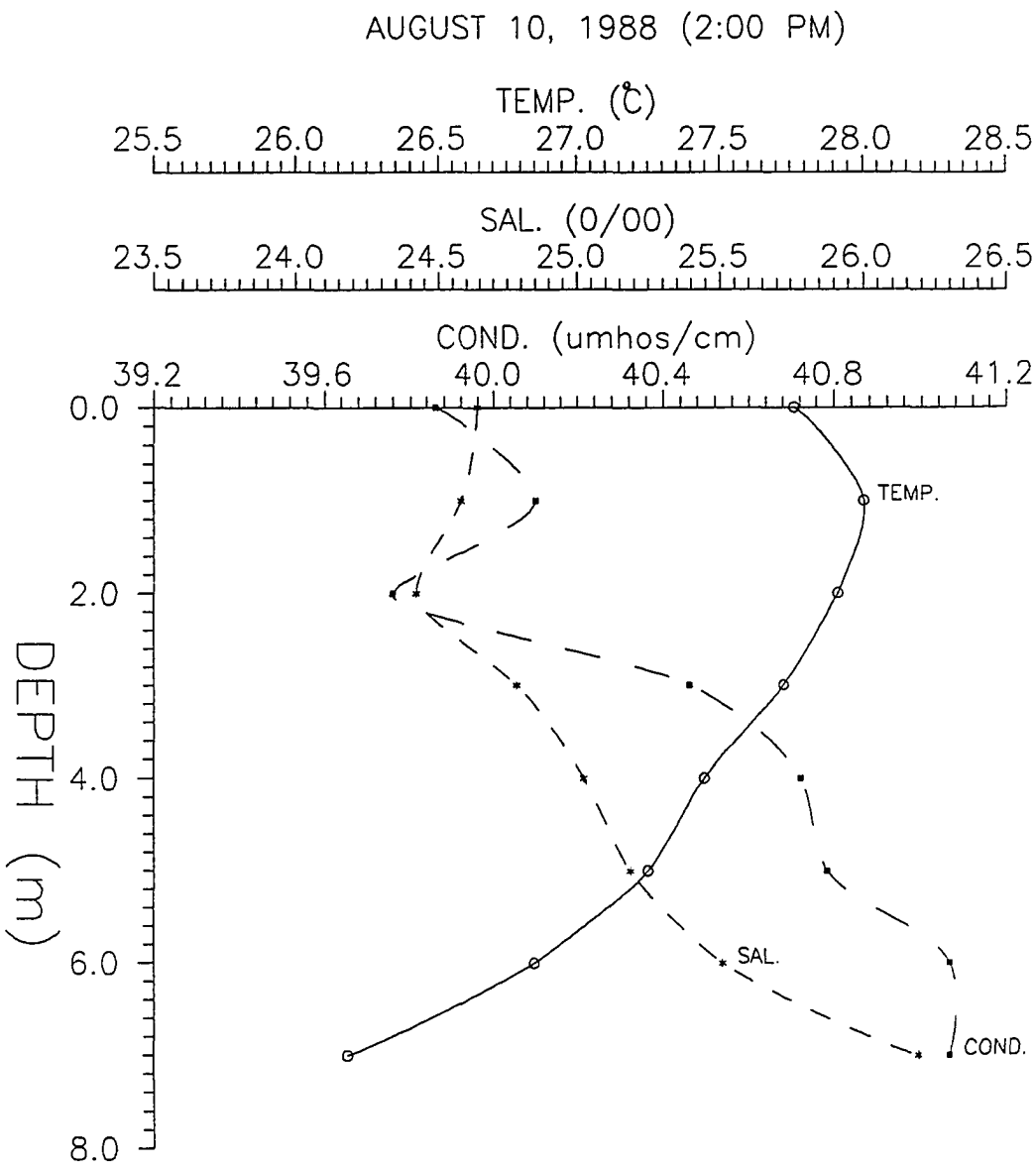
Appendix B. Physical Data for diel studies in August 1988 and January 1989. Measurements taken every meter throughout the water column. Temperature (circle); Salinity (asterisk); conductivity (square).

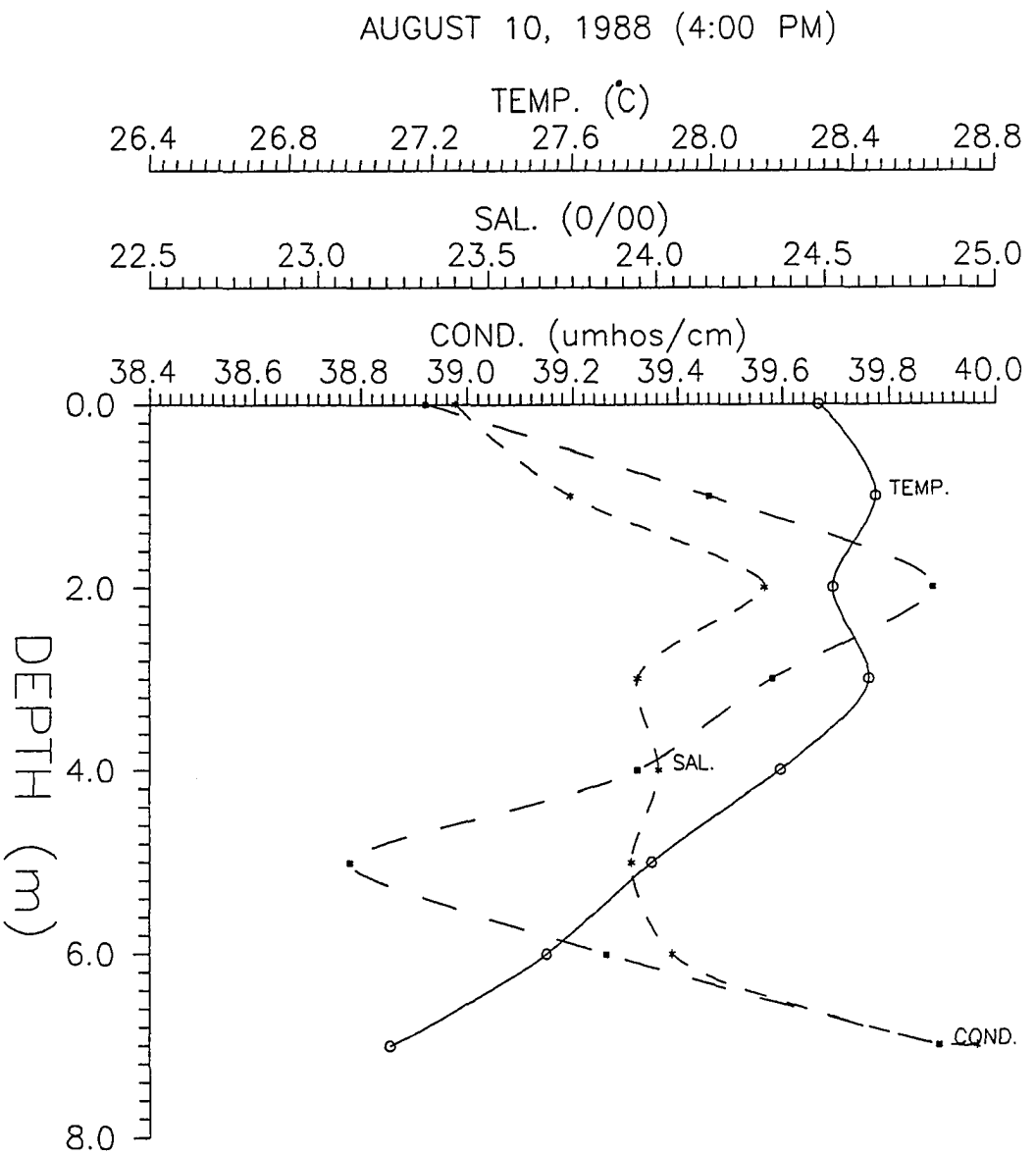


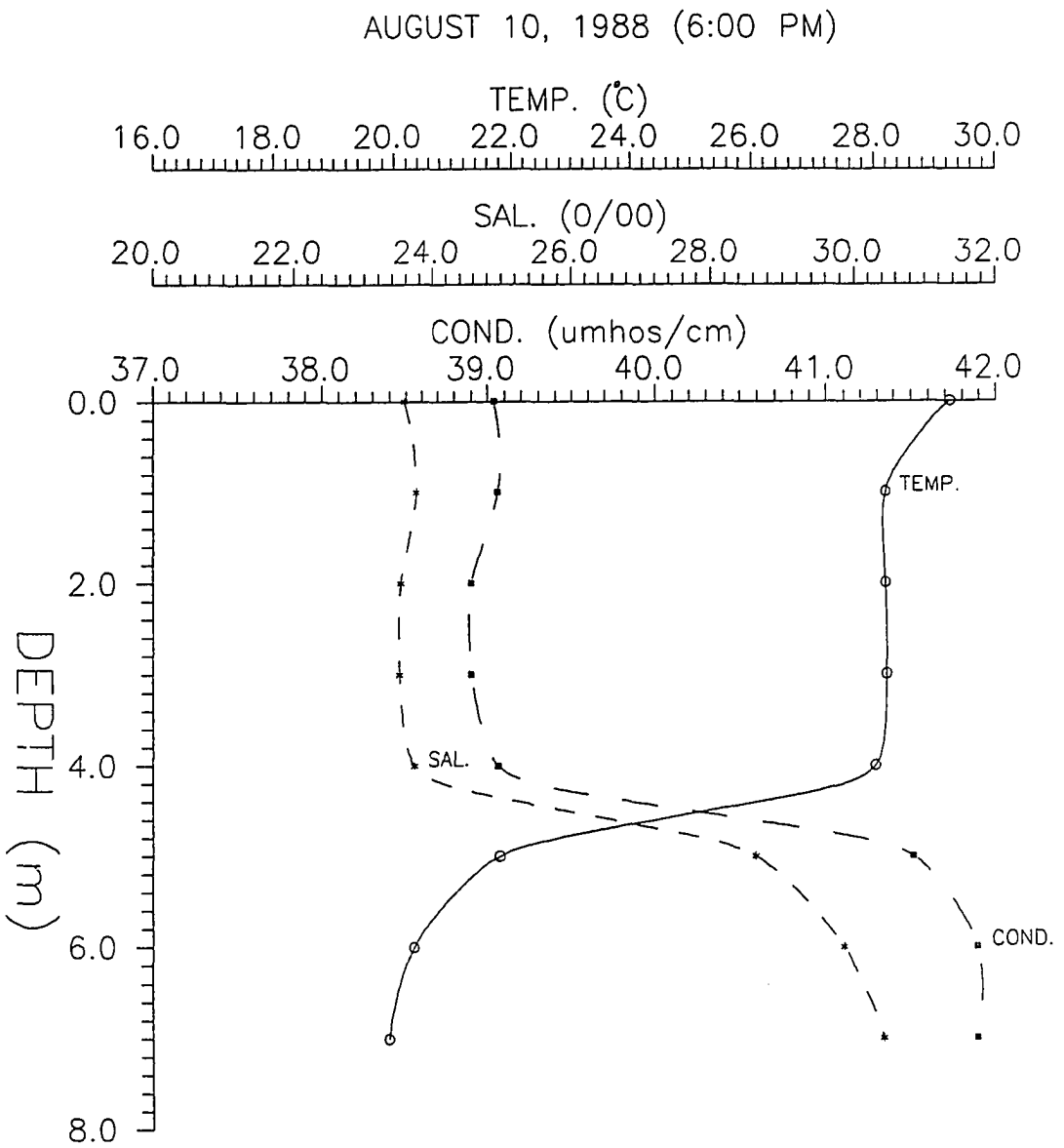


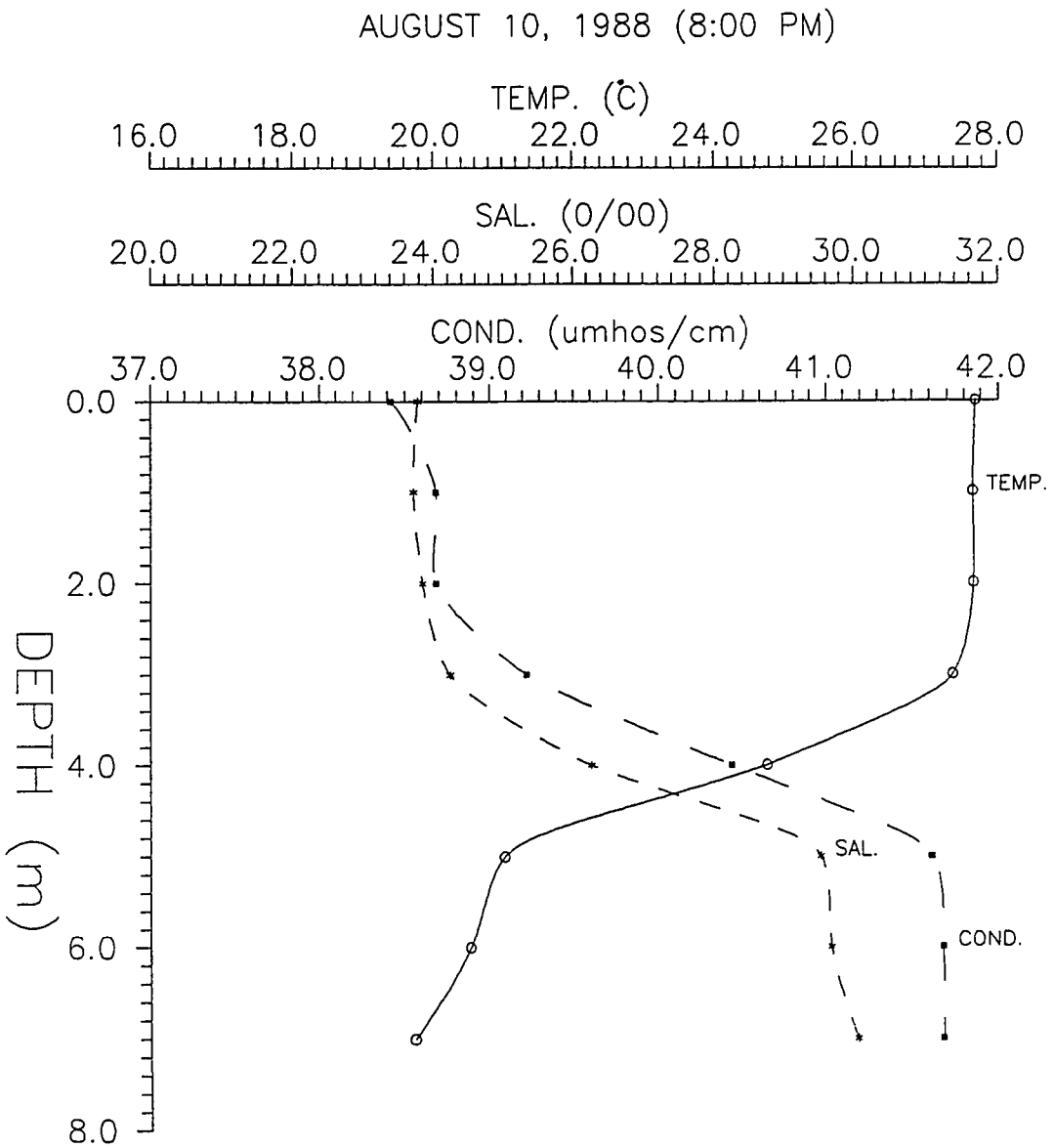


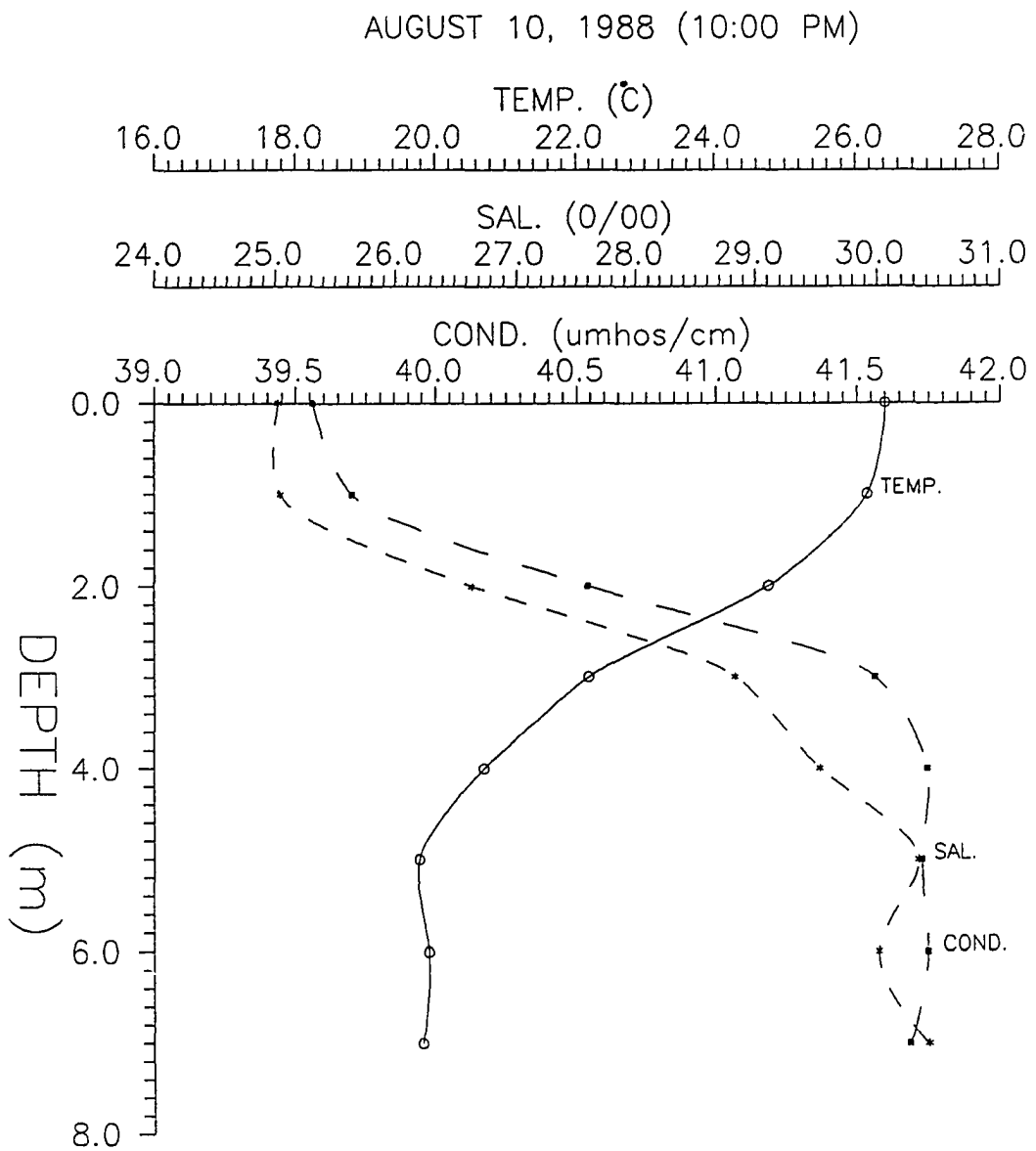


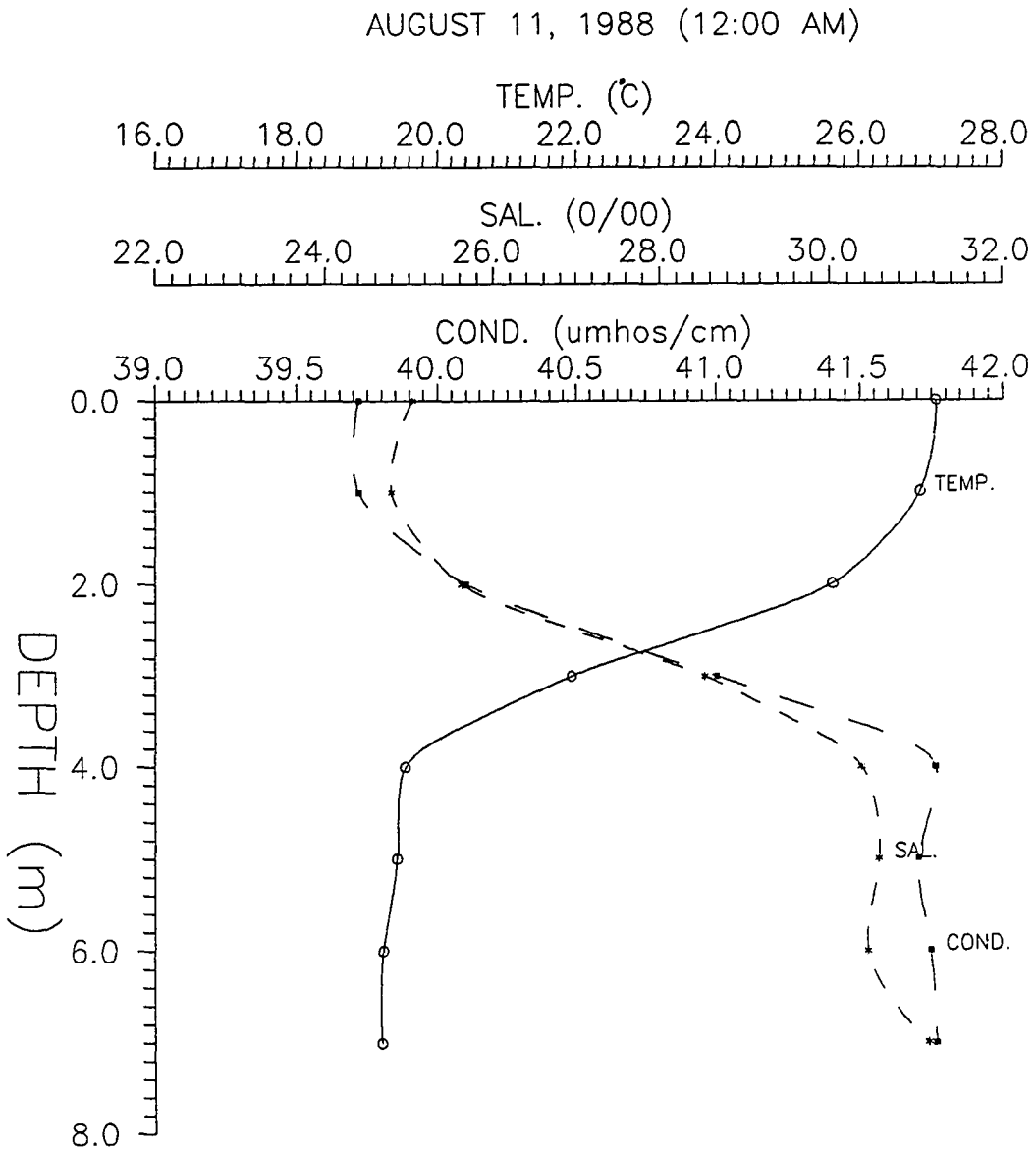


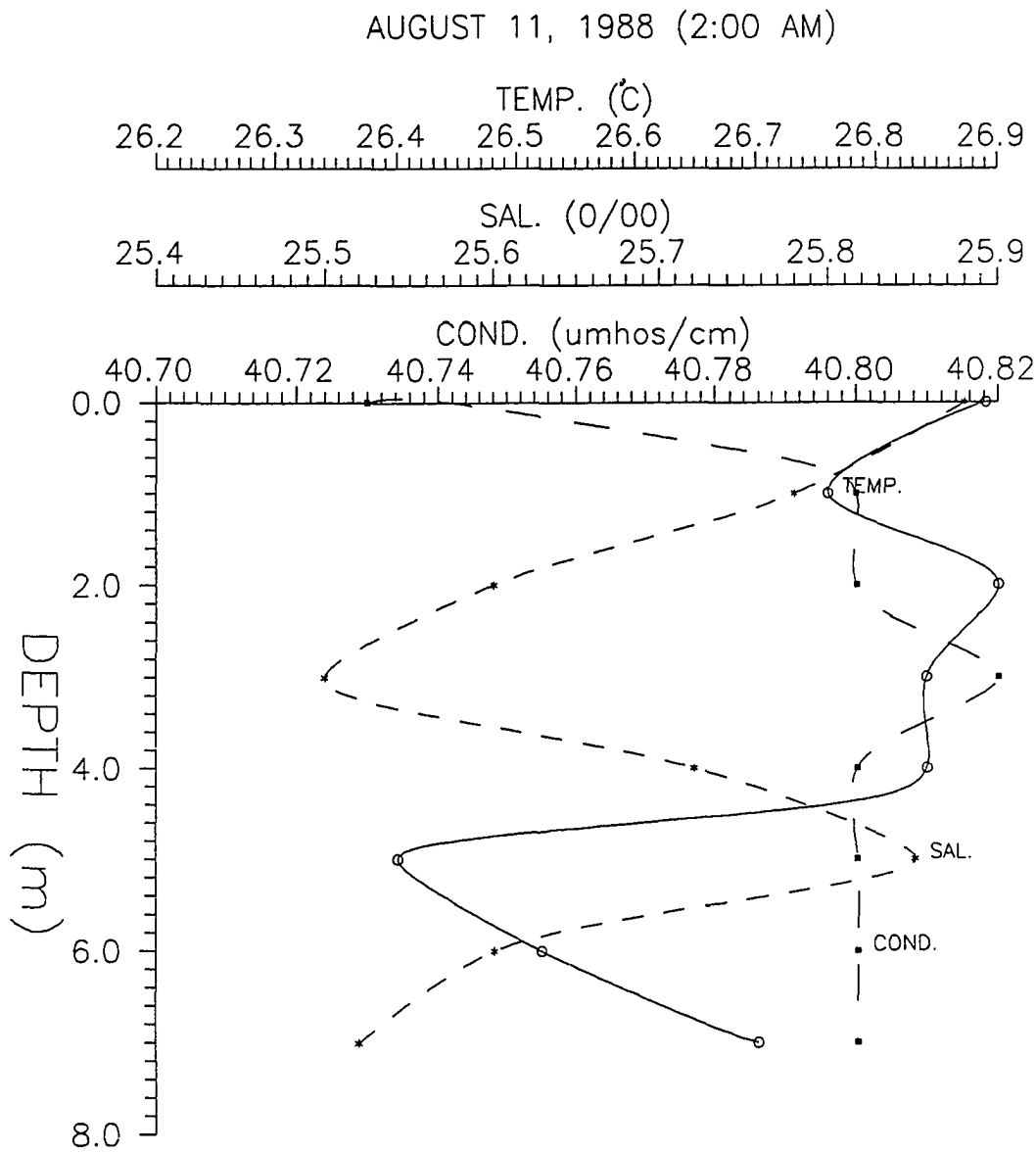


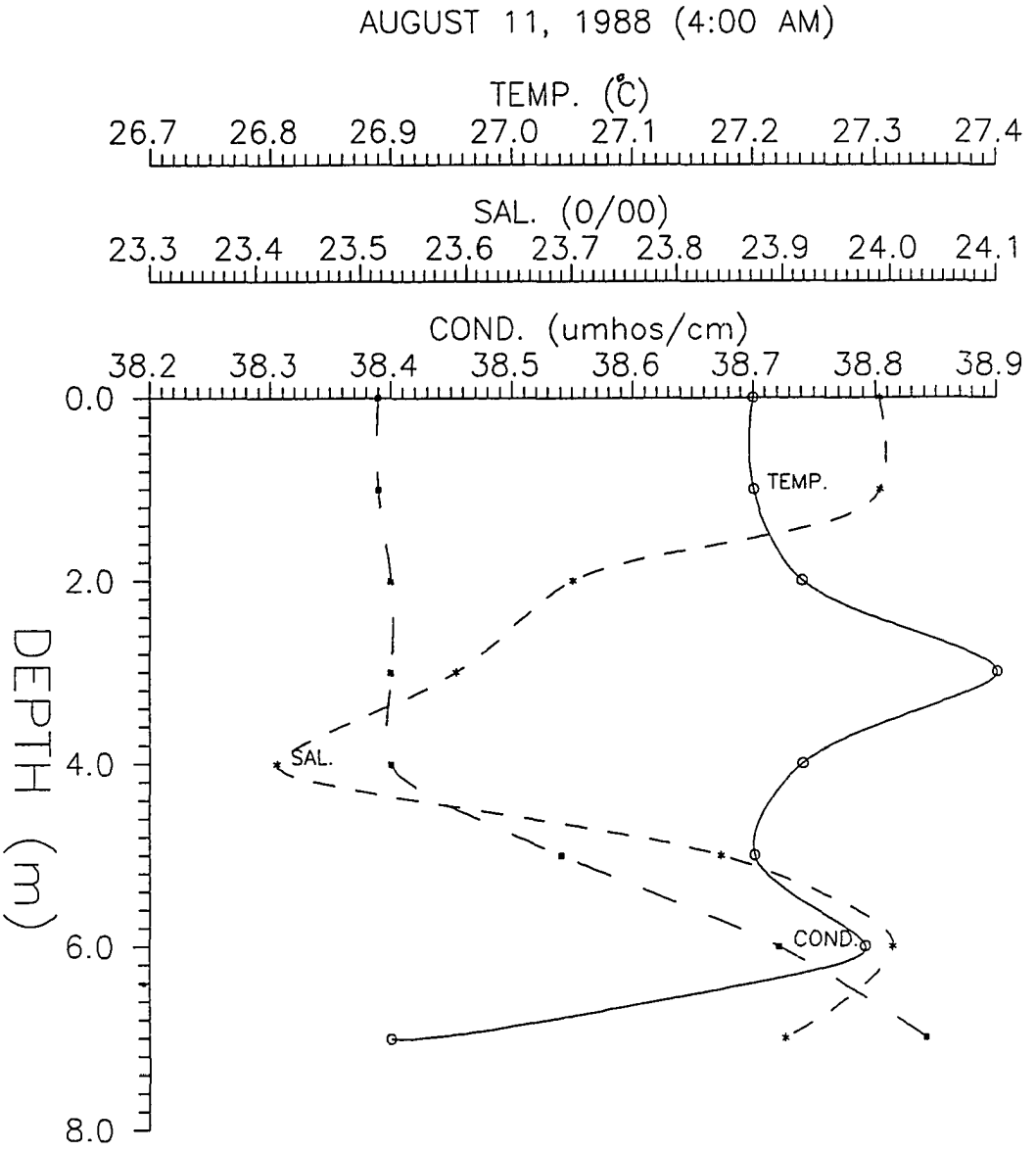


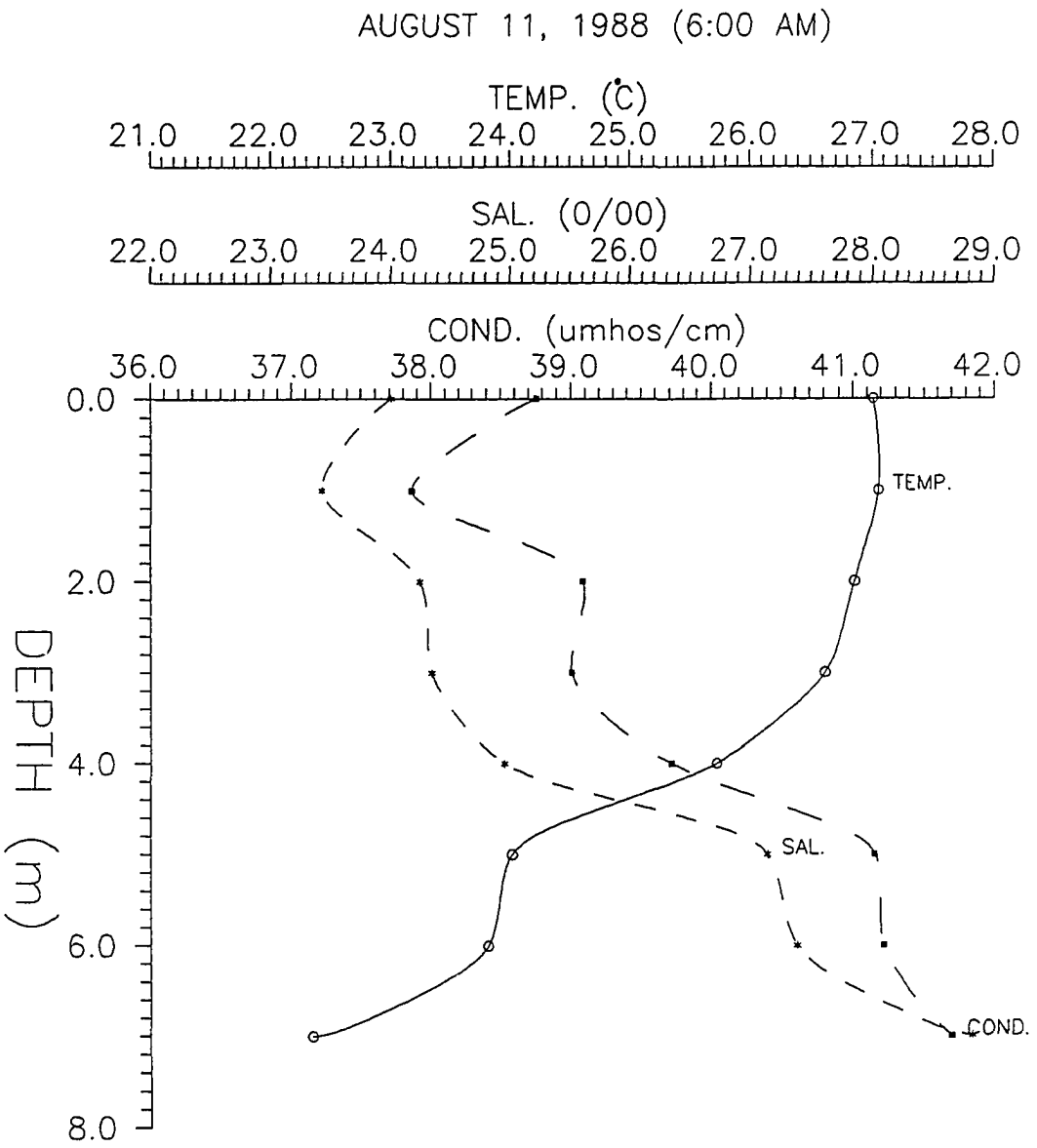


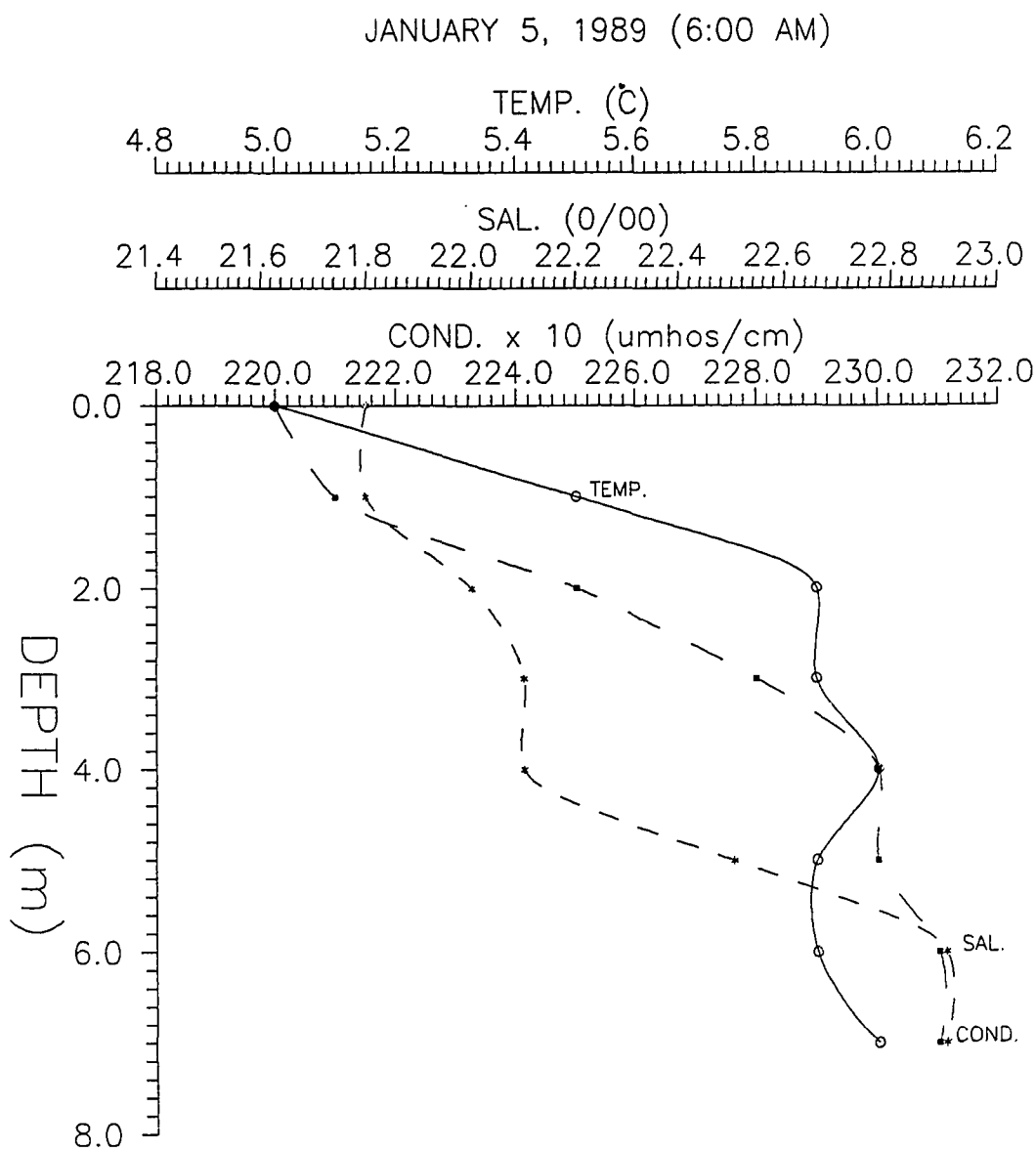


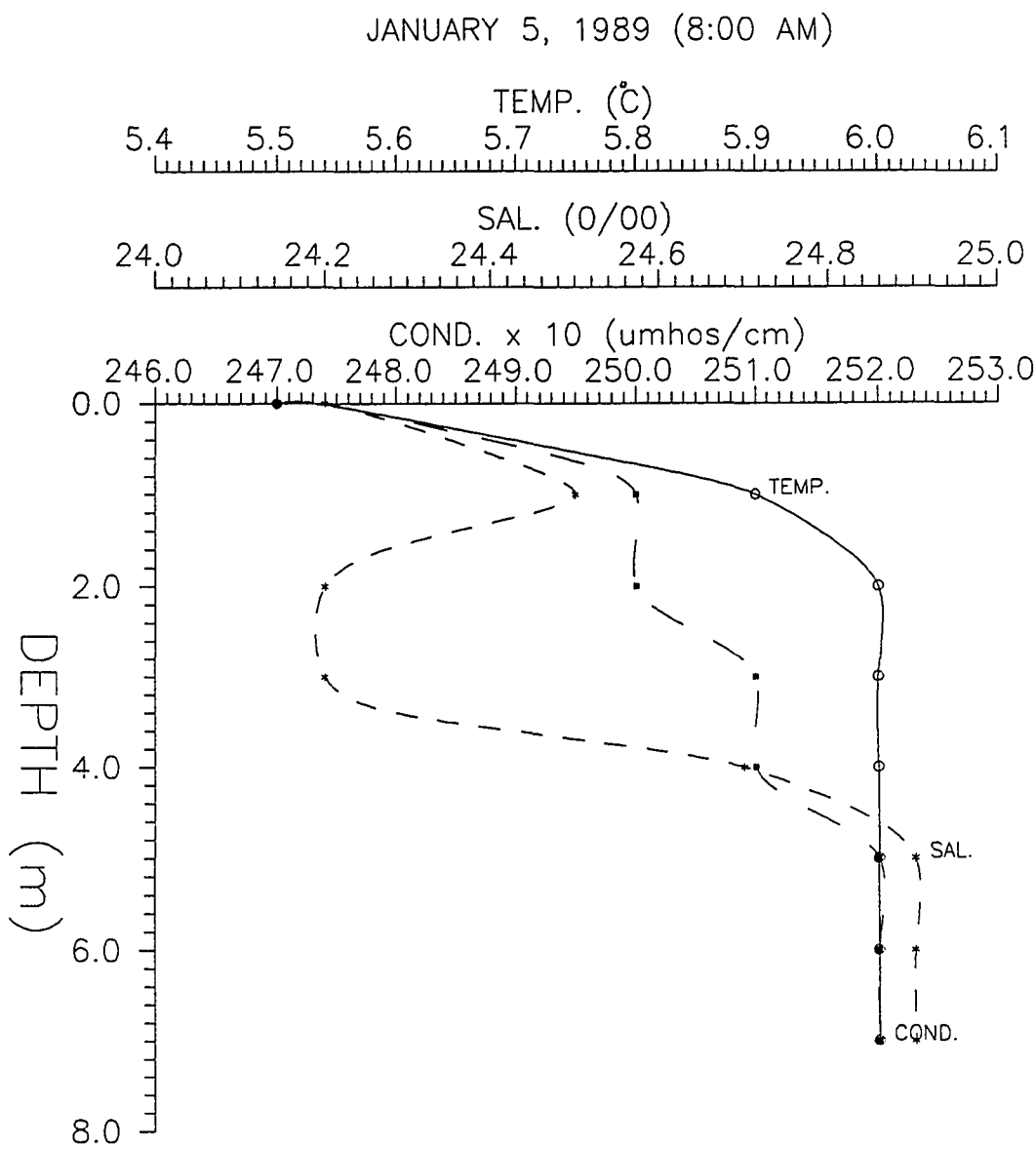


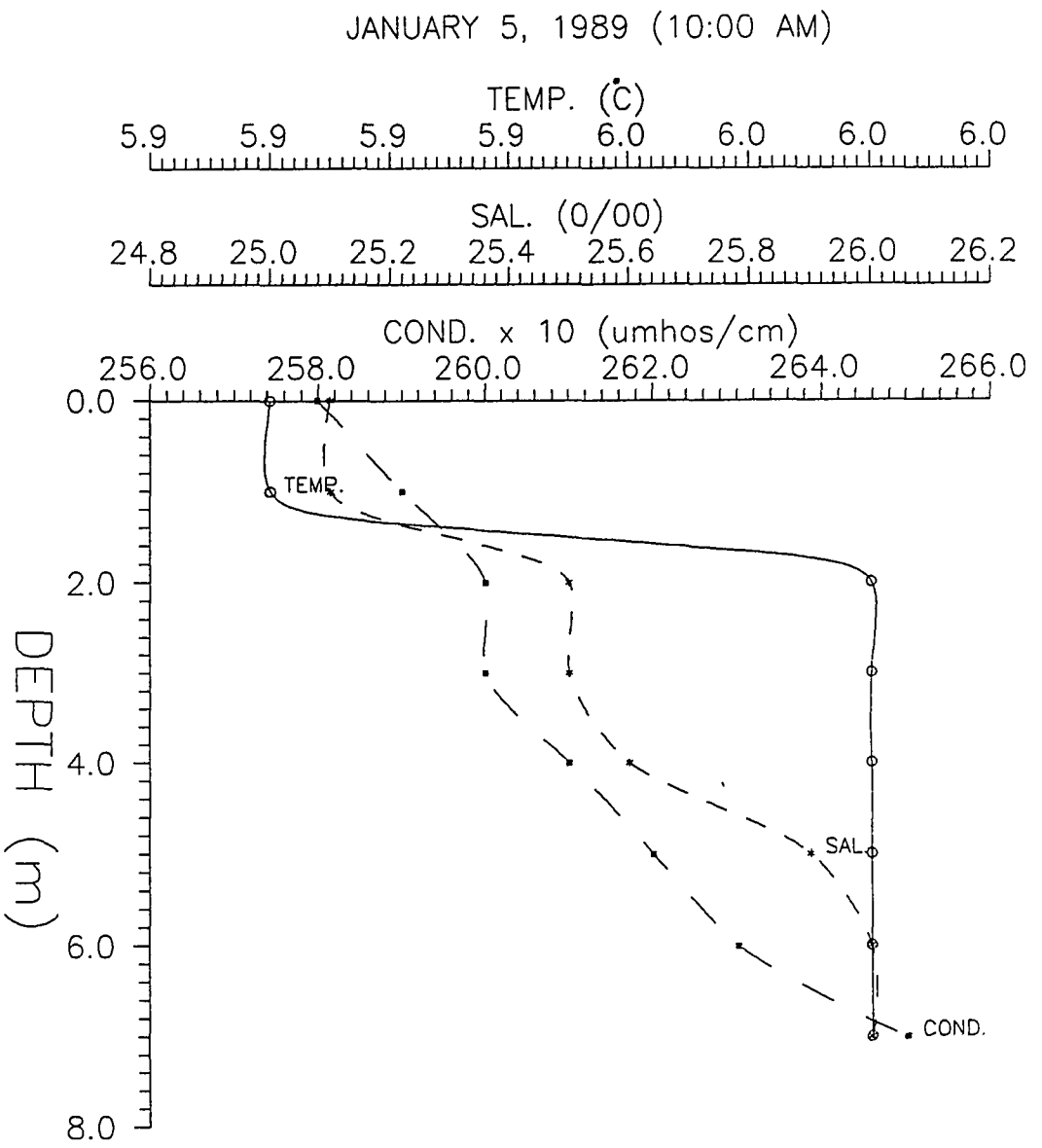


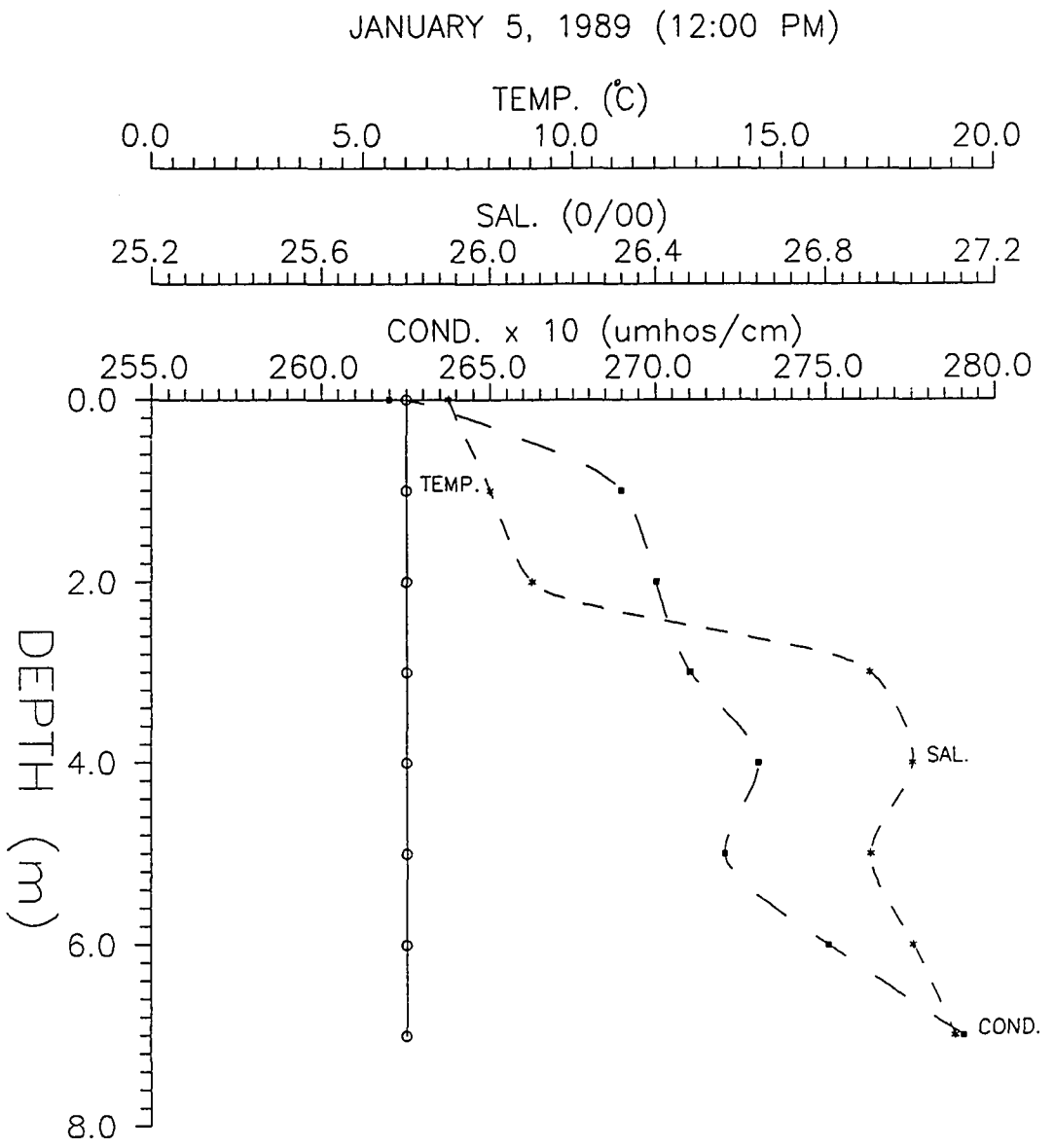


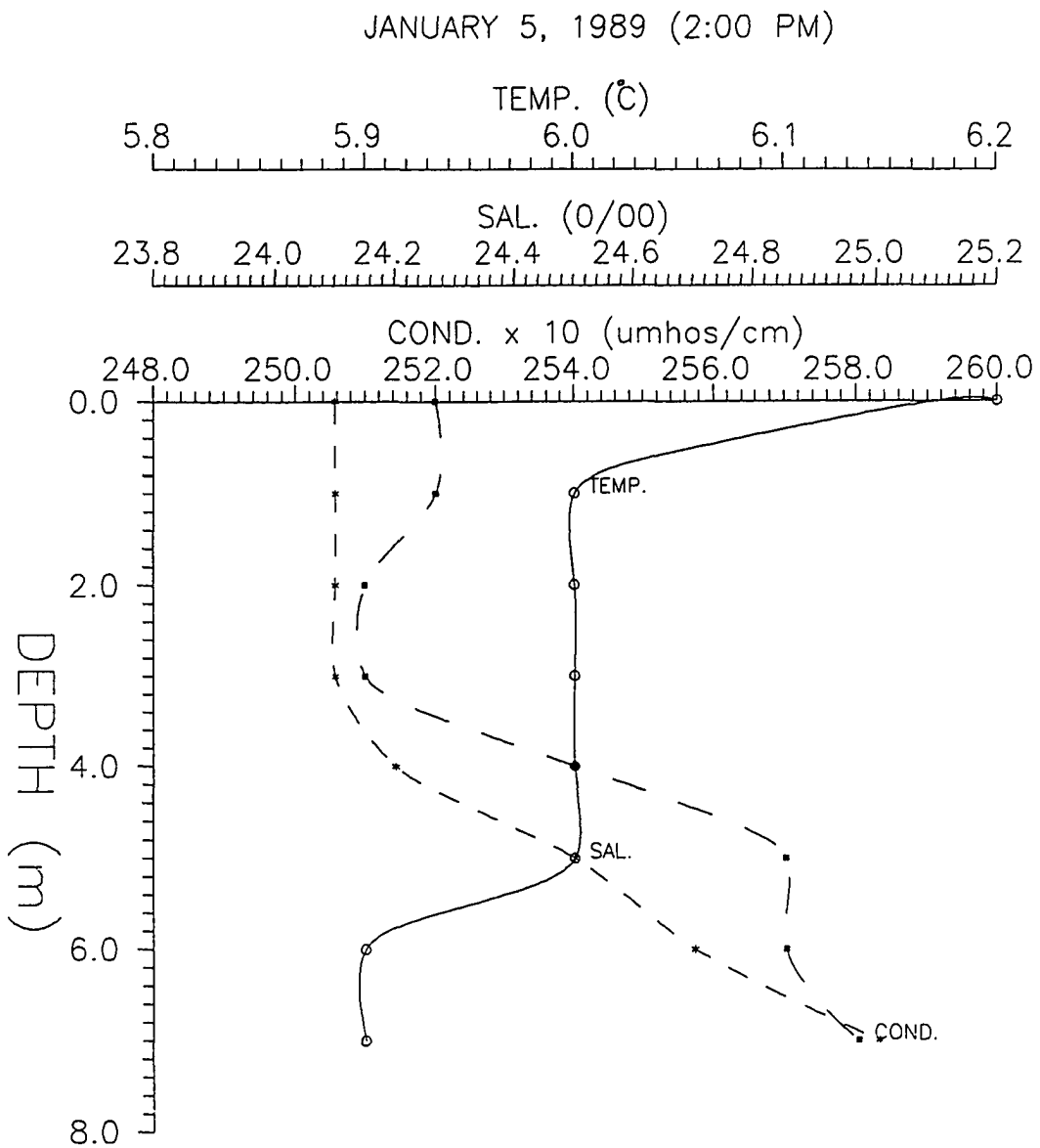


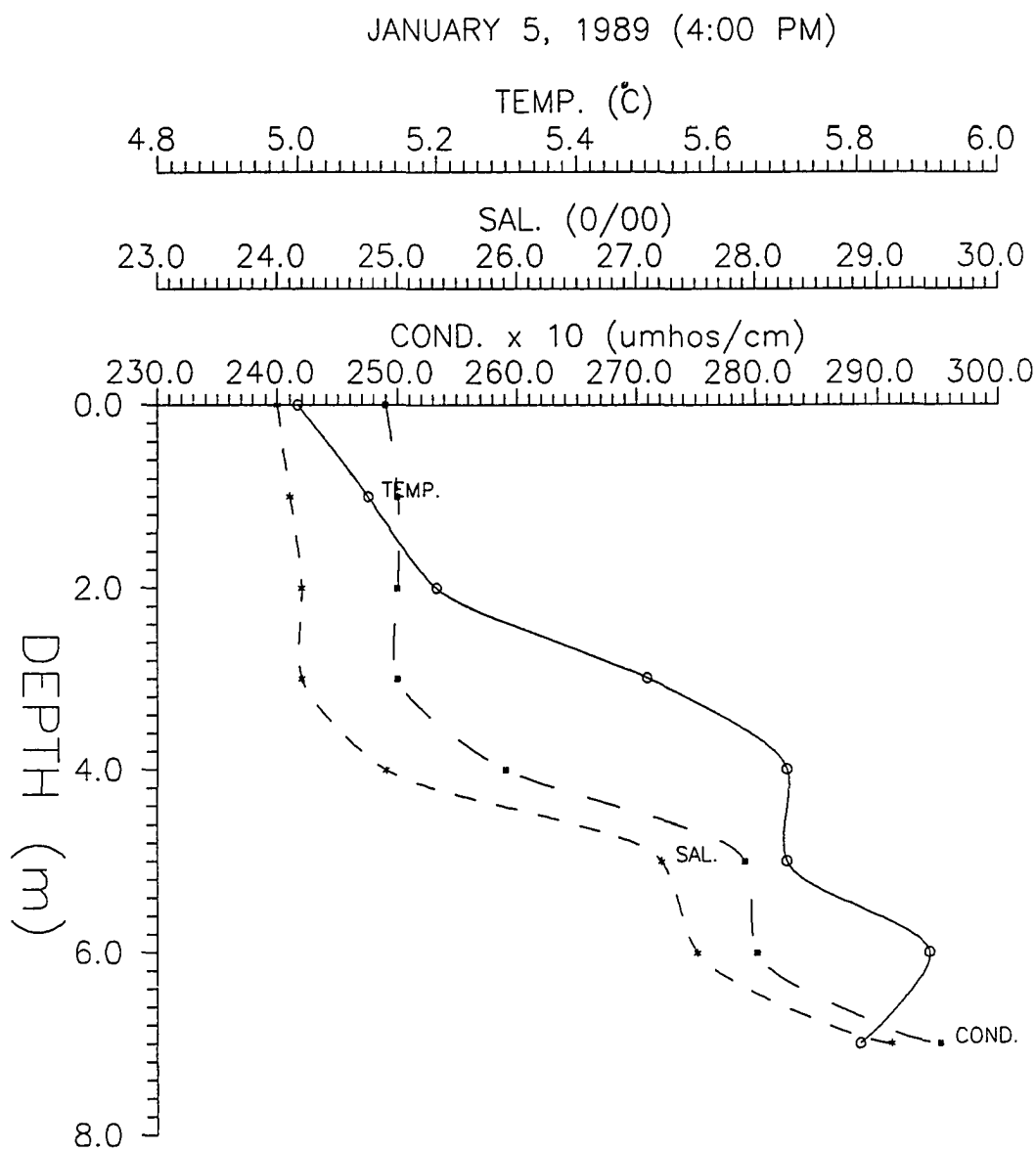


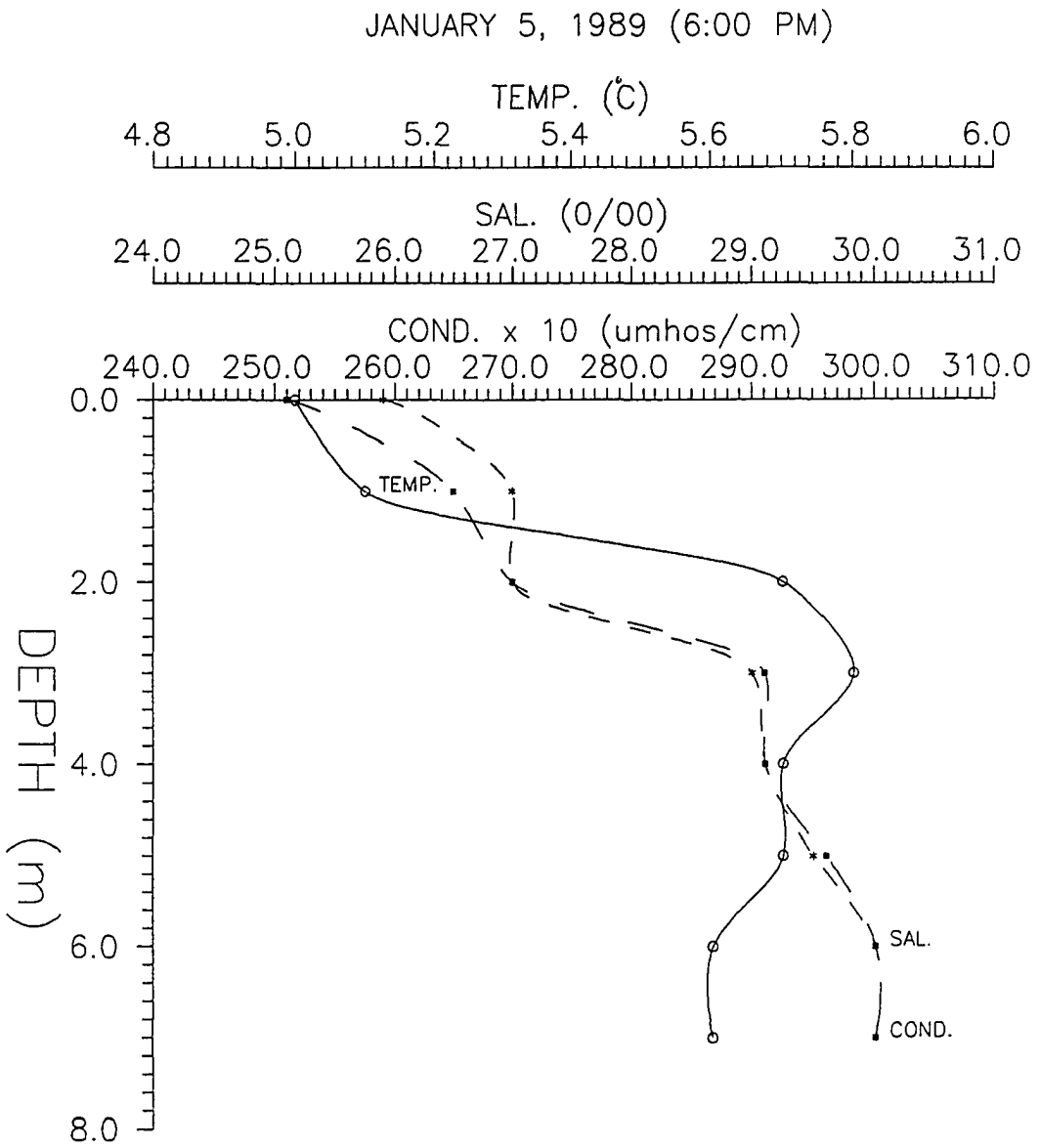


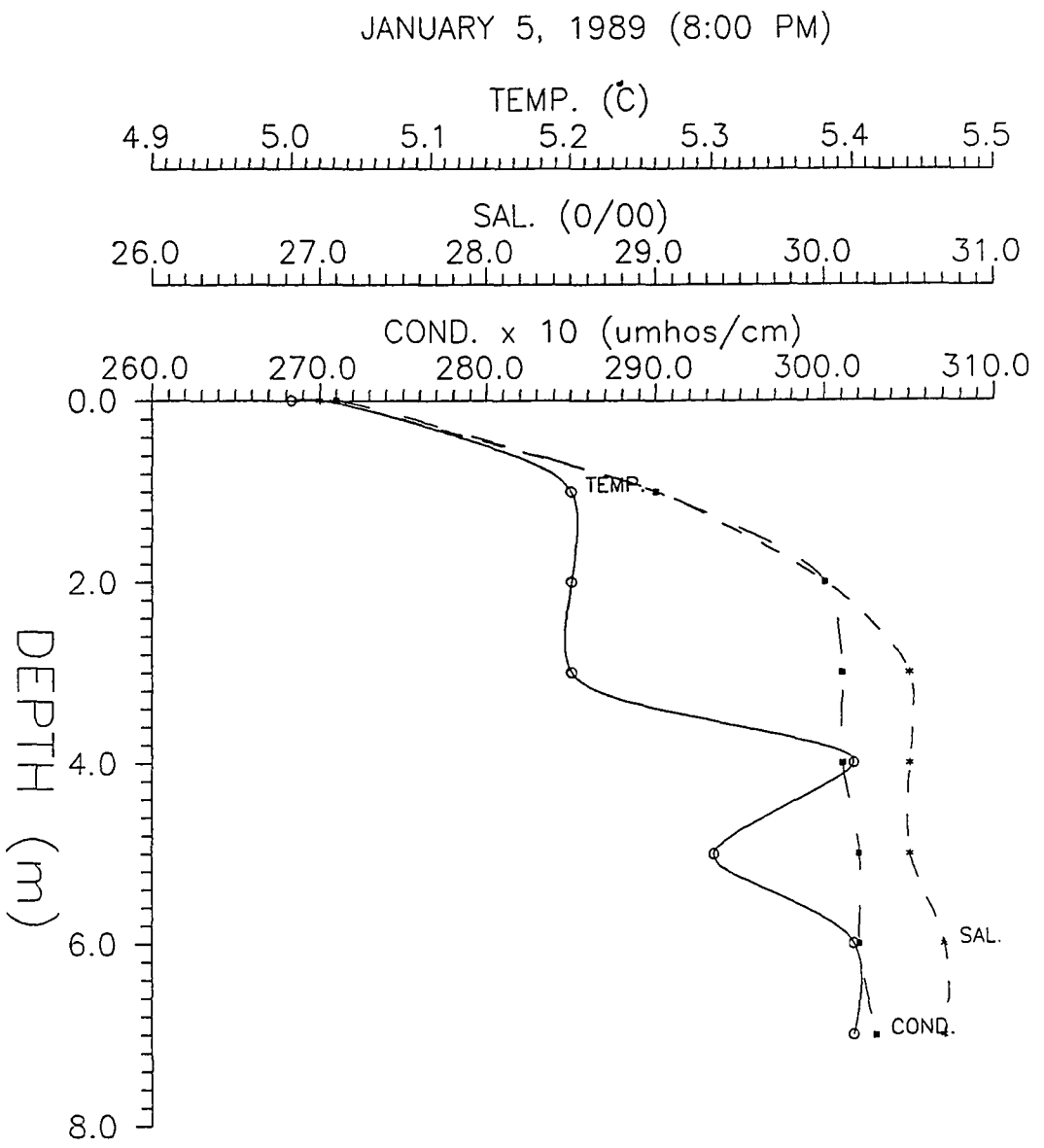


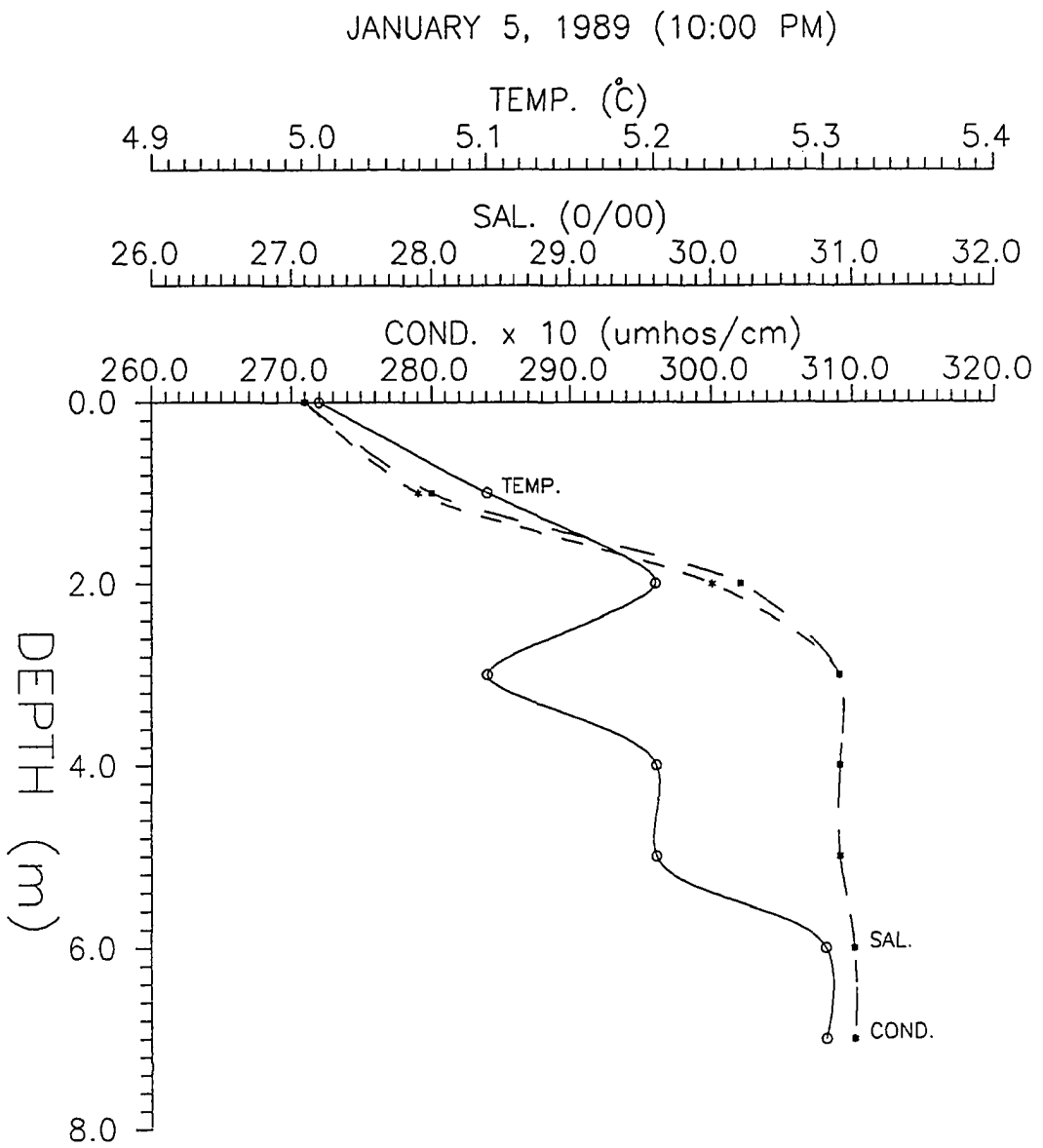


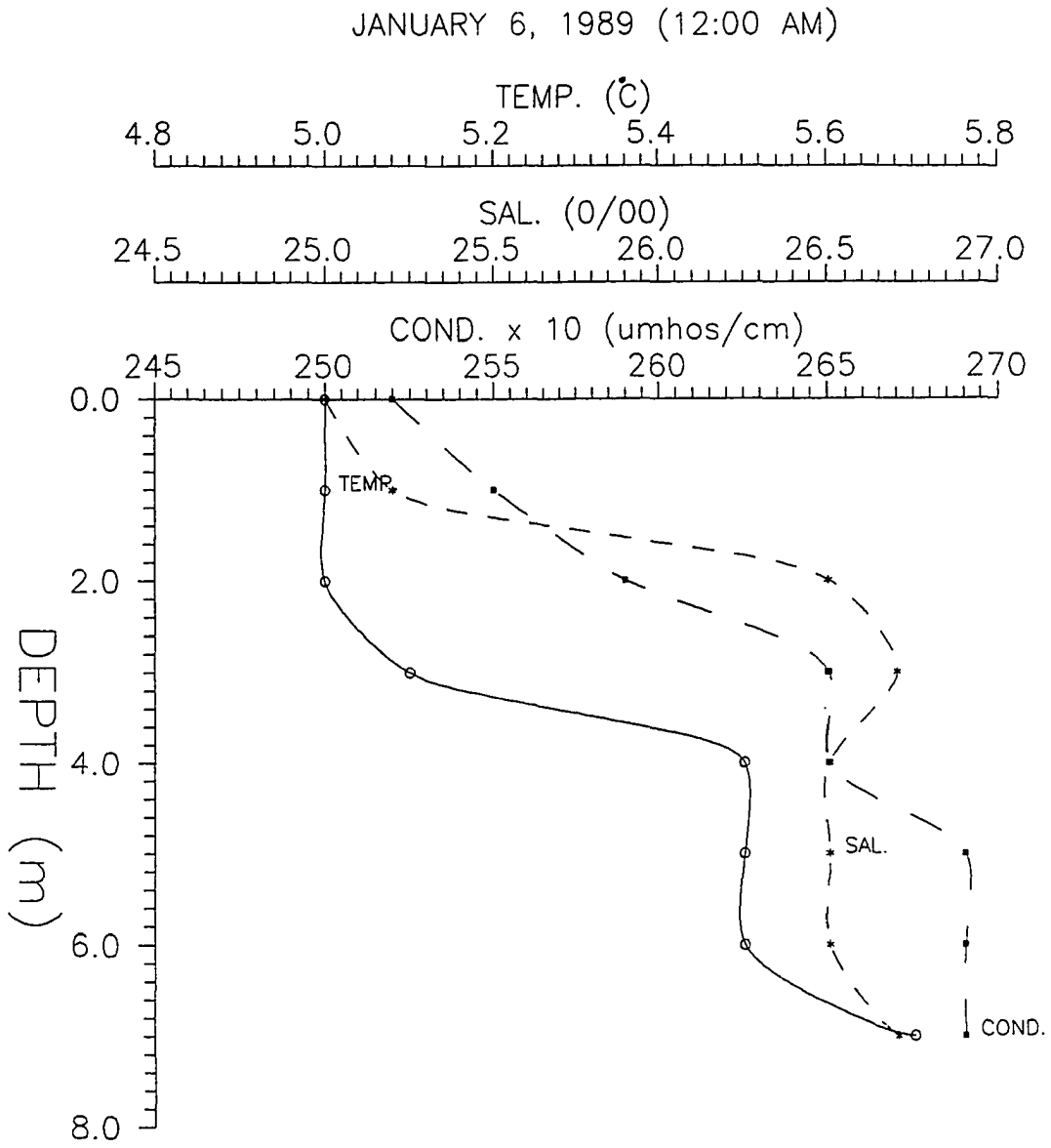


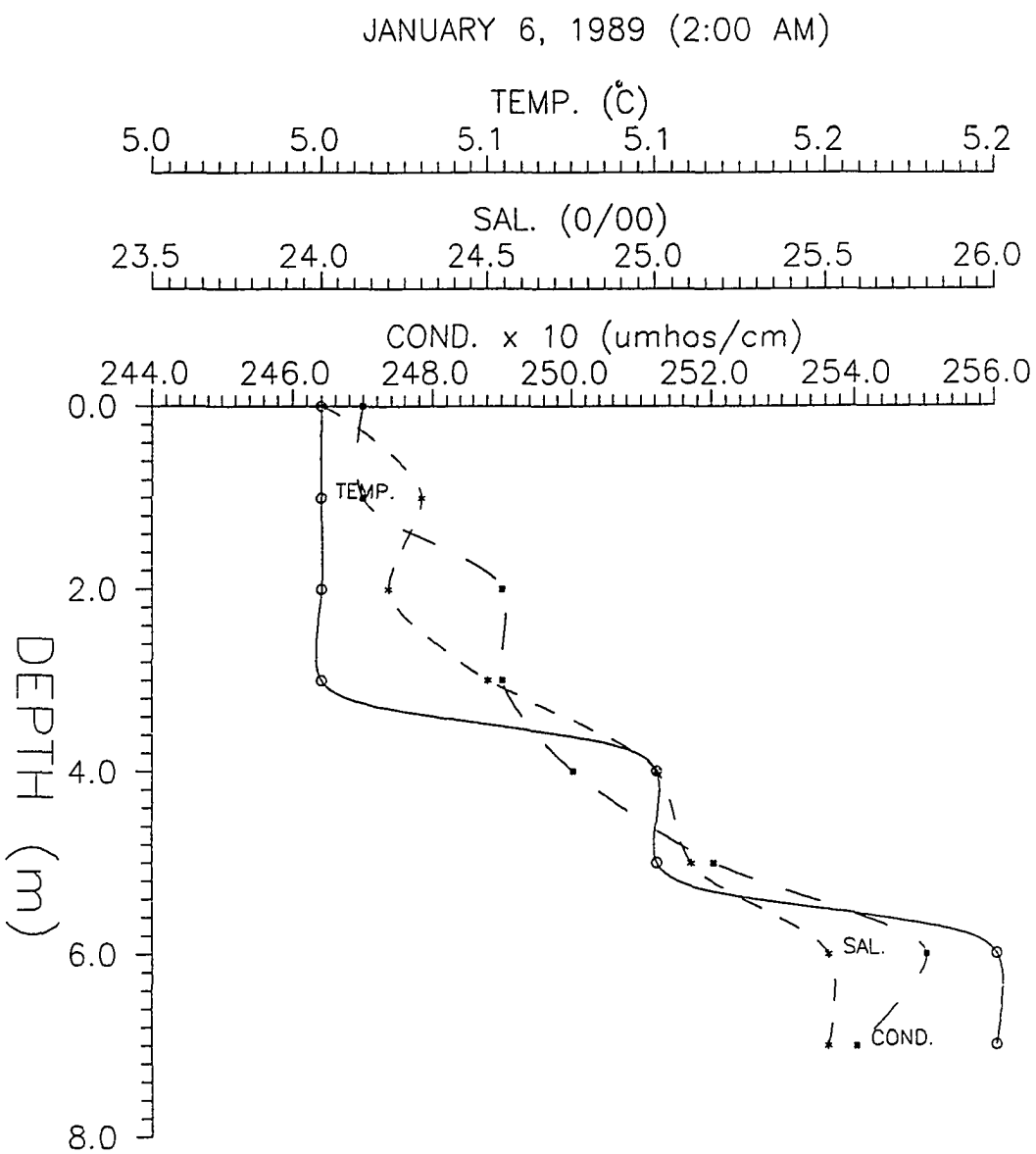


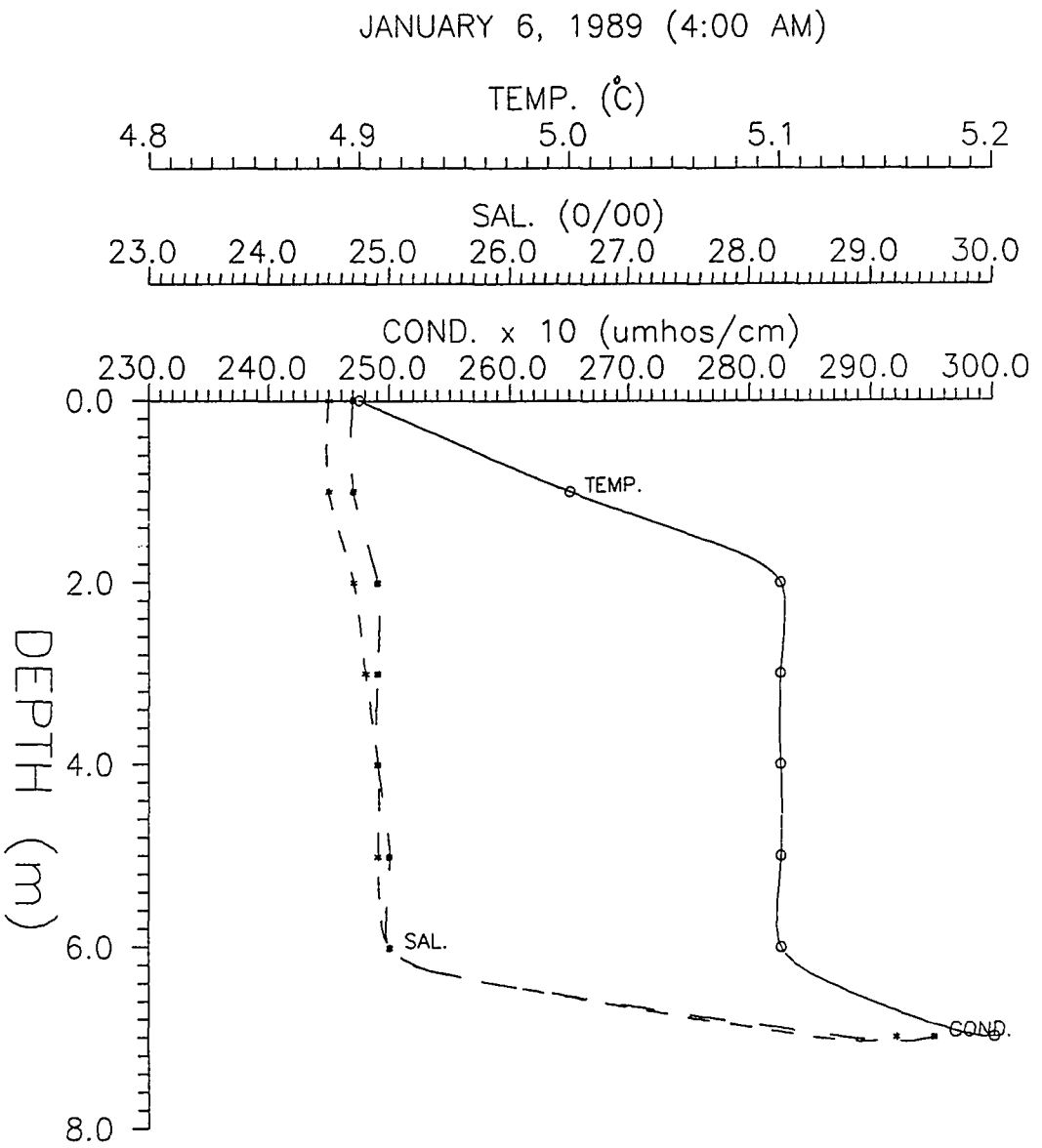


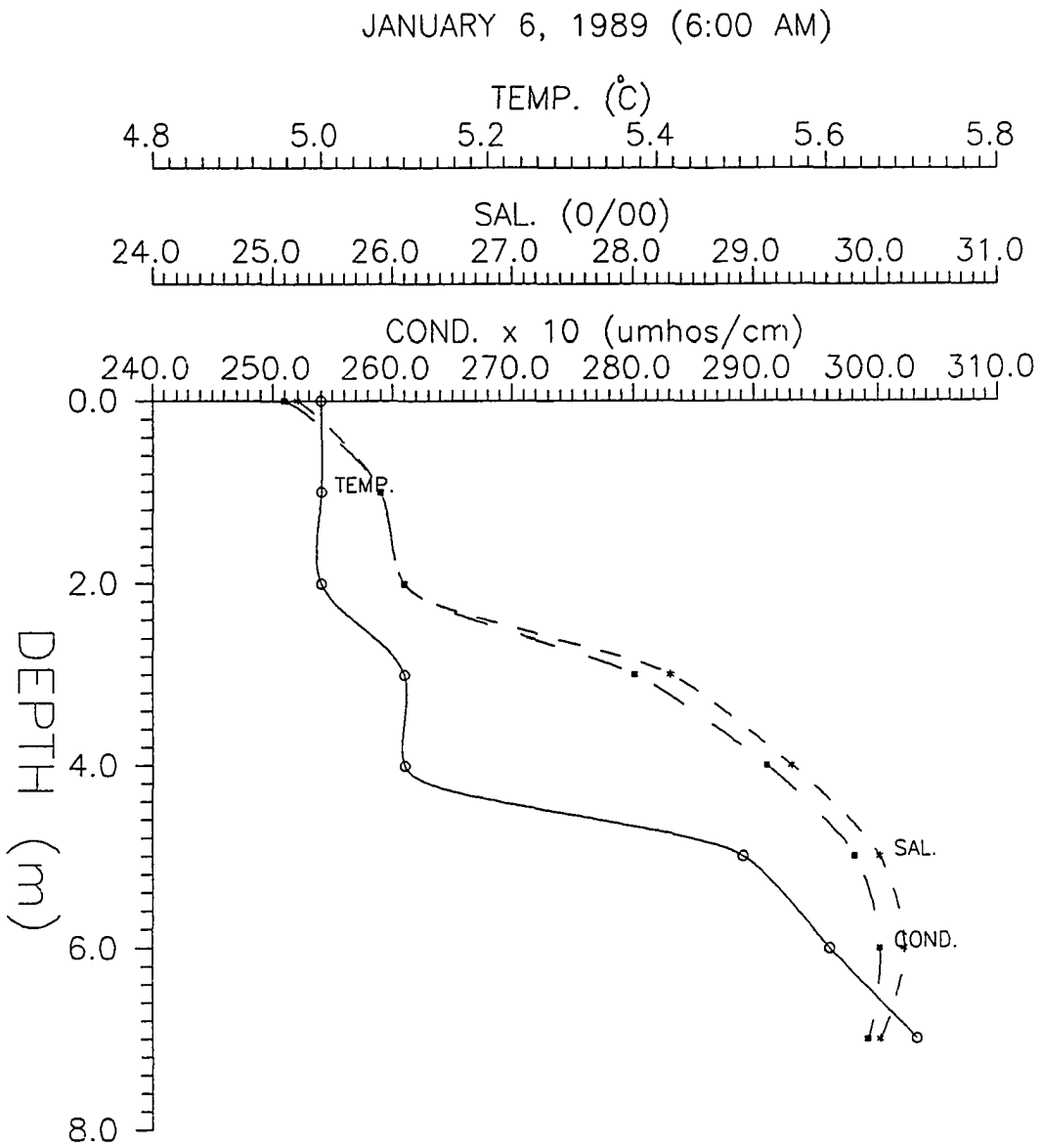




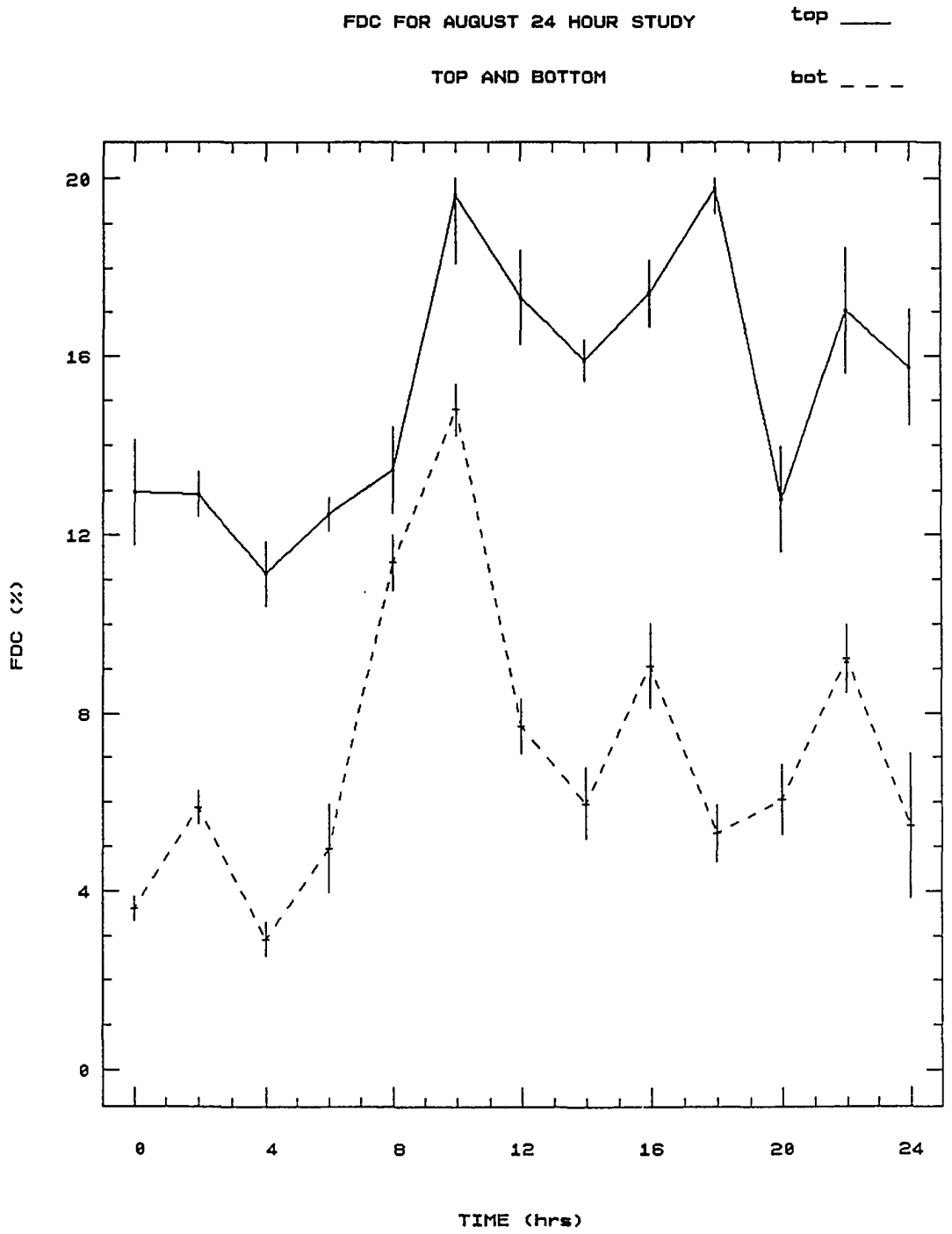


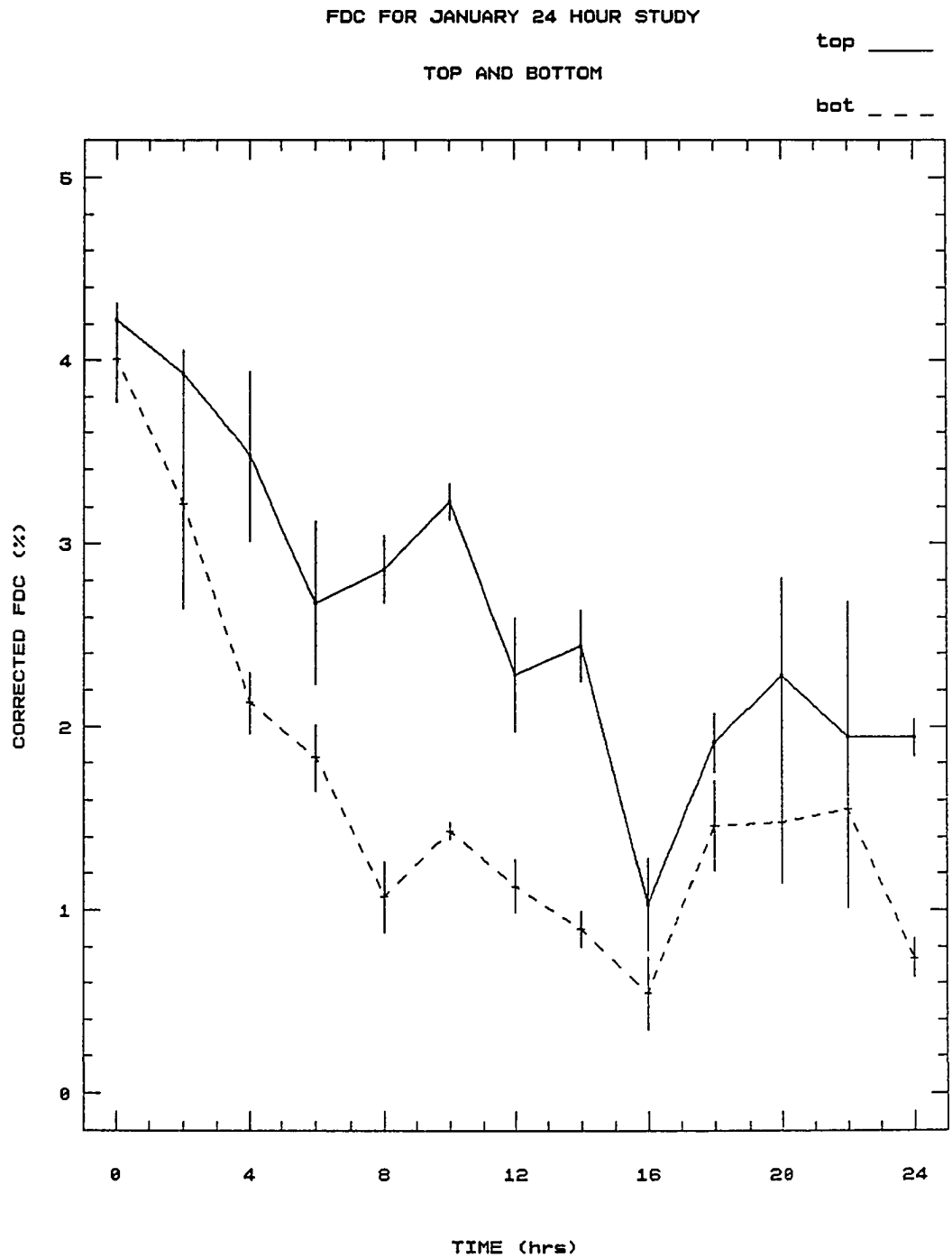






Appendix C. FDC values at both top and bottom sampling sites for August 1988 and January 1989 diel studies. Error bars represent the standard error calculated from three replicate samples.





AUTOBIOGRAPHICAL STATEMENT

The author was born in Atlanta, Georgia on December 29, 1958. He received his Bachelor of Science degree from Duke University in 1981 and a Master of Arts degree from The George Washington University in 1985. While finishing his Masters degree, he held the following positions:

> 1981 - 1984: Teaching Assistant. Northern Virginia Community College. Microbiology, Biology and Chemistry.
>
> 1984 - 1985: Instructor. Marymount College of Virginia. Microbiology.
>
> 1984 - 1986: Teacher. Fairfax County Public Schools, Robinson High School. Advanced Placement Biology, Chemistry, Head Cross Country and Indoor Track Coach.

While in the Ph.D. program in the Ecological Sciences at Old Dominion University, the author was a Research Assistant under Dr. Harold G. Marshall in the Old Dominion University Phytoplankton Laboratory and was involved in an internship program with the U. S. Army Corps of Engineers, Norfolk District.

He is a member of both Sigma XI and Phi Kappa Phi honor societies chapters of Old Dominion University.